1994

Evolutionary Patterns in Proteaceae Based on Comparative Floral and Inflorescence Ontogenies.

Andrew William Douglas
Louisiana State University and Agricultural & Mechanical College

EVOLUTIONARY PATTERNS IN PROTEACEAE BASED ON COMPARATIVE FLORAL AND INFLORESCENCE ONTOGENIES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Biology

by
Andrew William Douglas
B.A., College of Wooster, 1986
M.S., Illinois State University, 1989
December 1994

ACKNOWLEDGEMENTS

This research was supported by NSF Doctoral Dissertation Improvement Grant DEB-9213339.

I wish to thank Dr. Shirley C. Tucker, Dr. John Lynn, Dr. Marshall D. Sundberg, Dr. Russell L. Chapman, and Dr. Gordon Holcomb for their critiques, guidance, and support of this dissertation. I am also grateful to Dr. Philip T. Evans and Dr. G. Bruce Williamson who were present to support this research but unable to see the dissertation in its final form. I am very grateful to Dr. Marshall D. Sundberg who provided a unique space for the completion of this dissertation and allowed me to share with him his curiosity for plant forms and processes. I especially thank Dr. Shirley C. Tucker for her guidance, support, tolerance and for believing in me when others could not. As a guiding force in my young career, her contributions to my training and knowledge can best be summarized with a quote by John Gerard (1597, The Herball or Generall Historie of Plants) "*What our eies have seene, and hands have touched, we shall declare.*"

There are numerous people who have influenced this research. Dr. Lawrie Johnson, Dr. Barbara Briggs and Dr. Peter Weston have been extremely helpful as experts on the plant family. Others include Dr. Bernie Hyland, Dr. Ghilean T. Prance, Brett Hall, Dr. Betsey Jackes, Alf Salkin, Peter Jobson, Dr. Andrew Drinnan, Dr. Peter Stevens, Dr. Elizabeth (Toby) Kellogg, Dr. Douglas Soltis, and Dr. Mike Crisp. Still others have made it possible for me to sample a wide range of proteaceous taxa by taking the time from their busy schedules to collect material. These people include, Dr. Russell Chapman, Dr. Jennifer Chappill, Dr. Keith Ferguson, Bruce Gray, Tony Irvine, Bente Klitgaard, Dr. Tracey McClellan, and Dr. Margaret Sedgley. Barbara Keller of the Strybing Arboretum in San Francisco and Dr. Ray Collette, director of the

University of California, Santa Cruz, Arboretum were very helpful in providing permission to collect in the gardens.

Friends and colleagues who had an influence on this project include Dr. Elizabeth Harris (who provided the impetus to examine Proteaceae), Katherine Kantz, Marie Standifer, Dr. Tom Kantz, Dr. Martin Quigley, Dr. Terry Hedderson, Dr. Kevin Jones, Dr. Tom Wendt, Dr. Bill Odom, Dr. Anne Bruneau, Dr. John Constable, Dr. Mark Buchheim, Dr. Joey Spatafora, and Dr. Joseph Armstrong. I am especially grateful to the other friends I have gained during my tenure at L.S.U., Especially Rebecca Grouchy for her assistance with technical work.

Finally, I'd like to give my deepest appreciation to my lovely wife Ellen, who has shown me a side of life I never knew existed and has endured my passion for plants and the eternal quest of problem-solving. As a member of the Dead Plant Society, with her sarcasm, she has helped me to think of my research in truly innovative ways. I would also like to thank Toby, my daughter who has tolerated her early years with an absent-minded father. This research is dedicated to the Bad Banksia Men, the arch-nemeses of the Gum-nut babies, Snugglepot and Cuddlepie (May Gibbs, 1946). They really aren't bad, they're just drawn that way.

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ABSTRACT

Comparative floral and inflorescence ontogenies among Proteaceae are examined and a phylogenetic analysis based on principles of parsimony is presented. Comparative developmental and morphological investigations indicate that the four-merous flowers are primitively simple. Each flower is composed of: a single series of four tepals that are initiated in two dimerous whorls (in most taxa); four stamens, each one initiated opposite a tepal and generally fused to the tepal at various heights due to zonal growth; and a single carpel. Ontogenetic studies reveal that the nectaries arise late in development and are not homologous to a reduced petal or stamen whorl. The inflorescences of Proteaceae are racemose. Inflorescences among Grevilleoideae are more complex. The flower pairs of Grevilleoideae represent two flowers on a short-shoot; each flower is usually subtended by a floral bract, and the flowers are dorsiventral, with two tepals in both the median sagittal plane and the median frontal plane, like flowers in the other four subfamilies. The origin of the two-flowered short-shoots is either a result of reduction of secondary inflorescence branches or the product of an amplification event of first-order meristems along a primary axis. There are two forms of carpel initiation among taxa: a terminal transformation of the floral apex after stamen initiation, present in most taxa; in other taxa, the carpel is intiated in a lateral position and an apical residuum persists. Among grevilleoidean taxa, there are six orientations of the carpel conserved at different taxonomic levels. Developmental and morphological data are utilized in a phylogenetic analysis of 53 proteaceous taxa. Ontogenetic comparisons provide a clarified interpretation of floral diversity among taxa as well as a fundamental understanding of homology among characters in diverse taxa. The ontogenetic comparisons provided additional characters based on their timing and presence in the developmental program. The 154 characters provide a well resolved phylogeny of the family. The phylogenetic evidence does not support the currently

accepted classification of the family, as Persoonioideae and Proteoideae are not monophyletic and 14 of 17 tribes and 4 of 25 subtribes examined within the family are also nonmonophyletic.

CHAPTER 1

INTRODUCTION

Proteaceae is an isolated angiosperm plant family, isolated both morphologically and phylogenetically. Plants of the family are distributed primarily in the southern hemisphere with centers of diversity in Australia and the Malesian tropics, Africa, and South America. Proteaceous flowers are unique among other angiosperm plant families in being four-merous, having a single perianth whorl of four free tepals, four antetepalous stamens, with each stamen superposed opposite a tepal, and a single carpel. First classified by Brown (1810), Proteaceae has been reclassified by Johnson and Briggs (1975), but without resolving generic and suprageneric relationships. Johnson and Briggs (1975) identified a number of ambiguous features of Proteaceae that complicate assessments of homology. Some of the ambiguous features include: the single perianth whorl, hypogynous glands (nectaries), superposed or antetepalous stamens, inflorescence features and the paired flowers of Grevilleoideae, floral orientations, and carpel orientations. Johnson and Briggs (1975) pointed out that a better understanding of the ambiguous flower and inflorescence features within Proteaceae would ultimately provide the means to establish organizational similarities among taxa, and to improve classification.

A comparative ontogenetic study of flowers and inflorescences of Proteaceae was undertaken to clarify the developmental basis of the distinctive flowers. In addition, such a study would provide evidence of homology among floral organizations and provide developmental evidence of convergences and divergences involved in the evolutionary and floral diversity among the taxa.

This study ontogenetically compares 78 species representing 62 genera within Proteaceae and is intended to provide new evidence of the evolutionary diversity within the family. There are five objectives to this study: 1) to compare patterns of organogenesis and morphogenesis among taxa in Persoonioideae, the subfamily hypothesized as the basal group of the family (Johnson & Briggs, 1975); 2) to compare

divergent patterns of organogenesis and morphogenesis in flowers of Proteoideae; 3) to compare and examine the ontogenetic events that are responsible for the flower pairs in the subfamily Grevilleoideae, as well as to provide empirical evidence of the topological homology and orientations of the flowers in this diverse subfamily; 4) to compare and describe the ontogenetic events that result in diverse carpel orientations of proteaceous flowers, particularly among Grevilleoideae; and 5) to establish informative ontogenetic characters to be used in a phylogenetic analysis of the family and compare it with the classification by Johnson and Briggs (1975).

The first four comparative ontogenetic objectives could provide new evidence of the organization in diverse flowers among Proteaceae in relation to other angiosperms, as well as provide new evidence of the homology of characters among flowers and inflorescences within the family. Ontogenetic studies will thus provide detailed descriptions of the events that produce the topologically unique and diverse proteaceous flowers. They should contribute a better understanding of the patterns involved in flower and inflorescence diversity. These ontogenetic studies will also provide a unique context within which to examine phylogenetic relationships among Proteaceae in three ways: 1) Ontogenetic comparisons can help to establish homology by clarifying the nature of complex floral structures, since developmental stages are often easier to interpret. 2) Ontogenetic comparisons among taxa should reveal the developmental events that result in convergence or divergence of mature floral form among taxa. 3) Ontogeny can provide additional discrete phylogenetic characters that are not evident in the mature form.

Floral, inflorescence and phylogenetic comparisons within Proteaceae should prove significant, as they will clarify interpretation of the flowers in relation to those of other angiosperms, as well as elucidating the developmental events responsible for the morphological and phylogenetic diversity in the family.

CHAPTER 2

COMPARATIVE FLORAL ONTOGENIES IN SELECTED TAXA OF PERSOONIOIDEAE (PROTEACEAE)

INTRODUCTION

Proteaceae is a moderate sized family of approximately 75 genera and 1200 species. Members are found primarily in the southern hemisphere with the greatest abundance of species and genera in Australia and the Malesian region (Johnson and Briggs, 1975). The flowers of Proteaceae maintain a relatively consistent and unique structure composed of a single whorl of four free tepals, four antetepalous (superposed) basifixed tetrasporangiate anthers, (one borne opposite each tepal), and a single carpel. Johnson and Briggs (1975) divided the family into five subfamilies: Persoonioideae, Proteoideae, Sphalmioideae, Carnarvonioideae, and Grevilleoideae. Persoonioideae has been hypothesized to represent the basal clade of Proteaceae, based primarily on the unifying feature of diploid chromosome numbers (Venkata Rao, 1971; Johnson and Briggs, 1975). There are no unifying morphological characters defining Persoonioideae although Johnson and Briggs (1975) assert that different taxa of Persoonioideae maintain different, presumably, primitive characters. Persoonioideae is divided into two tribes: Bellendeneae containing the monotypic Tasmanian Bellendena montana (n=5) and Persoonieae that is divided into two subtribes: Placospermiinae represented by the monotypic Queensland endemic Placospermum coriaceum (n=7) and Persooniinae, comprising two genera (sensu Weston, 1983): Persoonia composed of over 30 species (n=7) in Australia and one species in New Zealand (n=14), and the monotypic New Caledonian Garnieria spathulifolia (n=7).

As part of an ongoing comparative project, the floral ontogenies of selected proteaceous taxa are being examined to determine the developmental basis of floral diversity within the family as well as to better understand the features of the highly canalized flowers. Evidence from comparative ontogenetic studies will ultimately be used to reexamine the classification of the family by Johnson and Briggs (1975). In this study, the floral ontogenies of representative members of Persoonioideae are

investigated and compared: Placospermum coriaceum, two species of Persoonia (P. myrtilloides and P. falcata), Garnieria spathulifolia, and Bellendena montana (partial developmental stages available).

METHODS AND MATERIALS

Taxa investigated and provenance are listed in Table 2.1. In all cases, fresh floral material was fixed in FAA (90 ml 50% ethanol; 5 ml glacial acetic acid; 5 ml 37% formalin). After fixation, material was stored in 50% ethanol. Floral material was microdissected in 95% ethanol with a Wild M2 dissecting scope using fiber optic illumination. Prepared materials were dehydrated with absolute alcohol and acetone and critical point dried using a Denton apparatus with liquid carbon dioxide. Dried materials were mounted on Aluminum stubs with colloidal graphite and coated with approximately 100-500 Angstroms of gold-palladium in either a Hummer II or Technics sputter coater. Prepared material was examined with a Cambridge S-260 scanning electron microscope. Images were recorded on Kodak Tri-X Pan 4 x 5 film. Developmental stages of Bellendena montana were taken from herbarium sheets. The material was rehydrated in approximately 75 ml warm water with two drops of dish-washing soap (Ivory, Proctor and Gamble, Inc.) for approximately 12 hours. The rehydrated material was rinsed in distilled water and stored in 50 % ethanol.

Materials used for histological study were dehydrated in an ethanol series. The plant material was infiltrated and embedded in "Histocryl" plastic according to the protocol provided (London Resin Co., Inc.). Sections were cut between 1.5 and 3.0 μm thick on a Sorvall JB-4 intermediate microtome and mounted on slides. Material was stained for 10-15 minutes in a 60°C oven in 0.5% Toluidine blue O in citrate-phosphate buffer at a pH of 4.5 (Sakai, 1973). Slides were rinsed in distilled, deionized,

Table 2.1. Names and provenance of species studied. **= species examined but not illustrated in text.

Taxa	Native to:	Source
Placospermum coriaceum C.T. White & Francis	N.E. Queensland	N.E. Queensland
Garnieria spathulifolia Brongn. & Gris.	New Caledonia	New Caledonia (Weston, coll.)
Persoonia myrtilloides Sieb. ex Schult & Schult F.	S.E. Australia	University of California at Santa Cruz (UCSC) arboretum
Persoonia falcata R. Br.	E. Australia	E. Australia, Queensland
** Persoonia mollis R. Br.	S. E. Australia	E. Australia (P. Weston, coll.)
** Persoonia oblongata R.Br.	S. E. Australia	E. Australia (P. Weston, coll.)
Bellendena montana R. Br.	Tasmania	Tasmania, (three different collectors, Alf Salkin, herb. material; Peter Jobson; Jennifer Chappill.

deoxygenated water to remove background staining. Images were captured with Kodak T-Max 100 4x5 film on a Nikon compound microscope.

OBSERVATIONS

Terminology - There are different terms associated with the different planes in flowers. Terminology that will be used include the median **sagittal** plane that bisects a flower, its subtending leaf (or bract) and the inflorescence axis. The median **frontal** plane is perpendicular to the median sagittal plane. The **adaxial** side of the flower is the upper half, closest to the inflorescence axis, and the **abaxial** side is the lower half of the flower closest to the subtending leaf (or bract). There are different terms associated with the symmetry of flowers. **Actinomorphy** is synonymous with radial symmetry, in which the flower has multiple planes of symmetry; **bisymmetry** refers to structures that have two planes of symmetry, that each bisect the structure or flower into two mirror-image halves (Weberling, 1989; Friis and Endress, 1990), and **zygomorphy** refers to structures or flowers that have one plane of symmetry; the abaxial and adaxial halves are not mirror-images (Weberling, 1989; Friis and Endress, 1990).

The structural homology of the single whorl of four free perianth lobes in Proteaceae to the perianth parts of other angiosperms is a source of controversy. Conflicting interpretations of the proteaceous perianth include: 1) homology to petals (Venkata Rao, 1971 - in part); 2) sepals persist after petal loss (Kausik, 1938, 1941; Eames, 1961; Haber, 1959, 1961, 1966; Venkata Rao, 1971 - in part); and 3: the flowers are primitively monochlamydeous as in pre-rosidalean taxa (Johnson and Briggs, 1975) (See discussion below). The perianth lobes are referred to as **tepals** because the perianth in different taxa can be petalline or sepalline in appearance. **Perigon** is the collective term for a perianth of equivalent segments (Johnson and Briggs, 1975; Weberling,

1989). The aestivation of the tepals in Proteaceae is valvate, that is, the lateral margins of neighbouring tepals are appressed and become interlocked via epidermal cell de-differentiation. Aestivation of the tips of tepals varies among some taxa. In most Proteaceae, the frontal tepal tips contact one another above the other floral organs. The sagittal tepals subsequently fill in the spaces between the frontal tepals and do not contact one another. This will be referred to as **incomplete-valvate aestivation.** Variations from this pattern are discussed when they occur.

The perianth lobes are diverse in form and structure among proteaceous taxa. A feature that is common among members of Persoonieae investigated here is a spine on the abaxial side of the tepals called a **Vorläuferspitze** (Baum, 1951). Preliminary anatomical investigations suggest that the spine is the vestige of the apical portion of the tepal.

Each of the four stamens in Proteaceae is antetepalous, borne opposite a tepal. Intercalary growth occurs between and beneath each tepal/stamen in most taxa resulting in epitepalous stamens (the stamen is adnate to the tepal). There are several terms associated with the morphological differentiation ot the anthers. **Median furrow** refers to the furrow or crease that bisects the longitudinal axis of each anther, resulting in two equal halves that become the thecae. The **transverse furrow** refers to the furrow or crease that divides each theca into two separate microsporangia (Kunze, 1978).

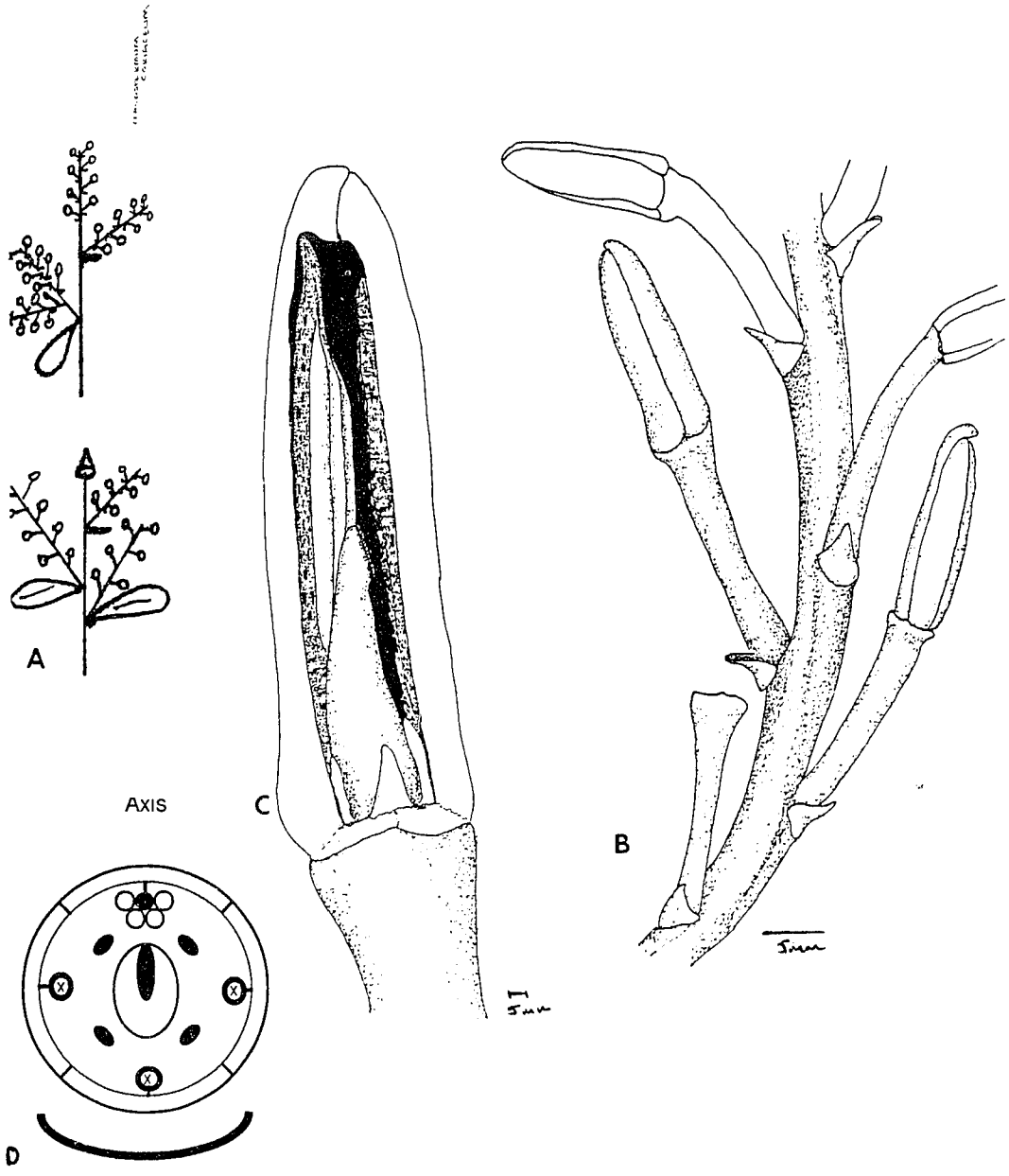
Flowers of Persoonioideae have a single terminal carpel. The entire floral apex, after stamen initiation, is utilized in the formation of the carpel. For the purpose of the following ontogenetic descriptions, the carpel primordium is initiated when the remaining floral meristem becomes zygomorphic after stamen initiation.

Inflorescences among members of Persoonioideae are variable in form. Terms for inflorescence morphology used here include **blastotelic**, i.e.indeterminate, versus an

anthotelic, i.e. determinate, axis ending in a flower (Briggs and Johnson, 1979). Briggs and Johnson (1979) differentiate between two types of blastotelic inflorescences: **anauxotelic** inflorescences are blastotelic inflorescences in which vegetative growth does not continue beyond the flowering region; **auxotelic** inflorescences are blastotelic inflorescences where vegetative growth continues beyond the flowering region. It should be noted that auxotely and anauxotely are applicable to a shoot axis as well as parts of inflorescences (Briggs and Johnson, 1979; Grimes, 1992). A **relative growth unit** is the axis produced by a single meristem (Grimes, 1992). Floral bracts are generally reduced leaf homologues and are referred to as **pherophylls** (Briggs and Johnson, 1979). In some Persoonia species, the subtending organ is a leaf or an unreduced pherophyll.

Persoonieae: Placospermiinae - **Placospermum coriaceum** - Organography - (Figs. 2-1-24). Inflorescences are found in the axils of serially transformed leaf/bracts towards the distal ends of leafy branches (Fig. 2.1A). The shoot relative growth unit (RGU) iss auxotelic, the vegetative growth occurring beyond the flowering portion. On some branches the shoot terminates in an inflorescence (anauxotelic). In most plants, secondary racemes (axes) develop from the lower portions of the primary inflorescence axis. Each flowering branch (axis) is a loose raceme having elongate internodes between each of the six to twenty cuneate bracts (Fig. 2.1B) (versus a condensed raceme that has little internode elongation). Each bract subtends a single flower. On lower portions of each flowering axis, the flowers are functionally male, each bearing a reduced and sterile carpel. Other flowers are perfect. Crimson red flowers are attached to long pedicels and, at anthesis, the distal portion of the tepals reflex slightly. There is a slight abaxial curve in the mature perigon. At anthesis, the flowers are highly zygomorphic (Fig. 2.1C) in the form of the perigon, in the single fertile stamen in the adaxial half of the flower and in the stigmatic area that differentiates on the distal

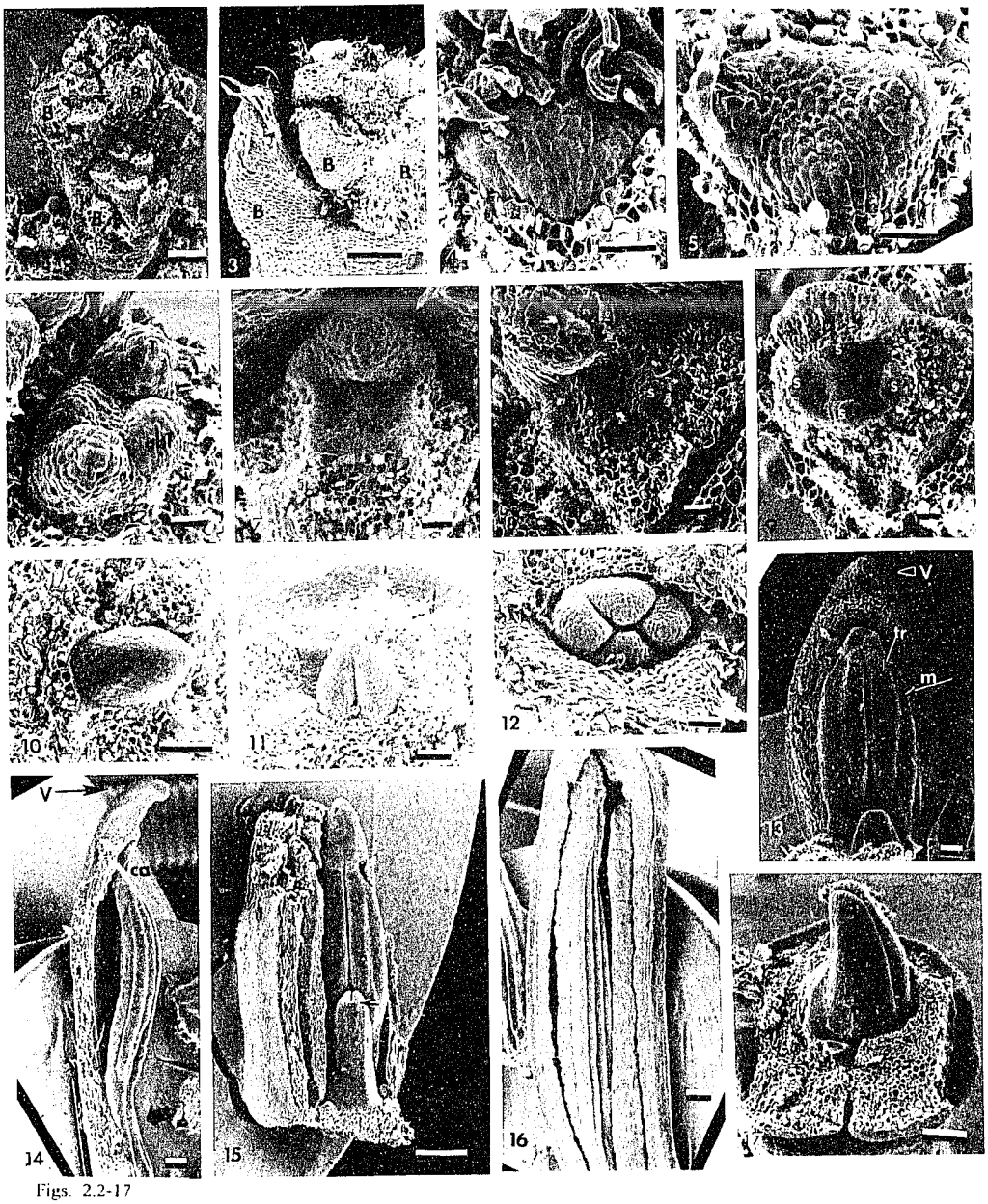
Figs. 2-1A-D. Floral and inflorescence diagrams of Placospermum coriaceum. **A.** Two types of inflorescence position observed on different branches, terminating with an inflorescence (auxotelic) above and axillary racemes in leaf or bract axils (anauxotelic) below. **B.** Camera lucida drawing of portion of raceme showing flowers attached to long pedicels and subtending cuneate bracts. **C.** Floral diagram showing arrangement of tepals (T), stamens (A), carpel (C) and nectaries (N). Adnation between tepals and stamens is present (-). **D.** Longitudinal camera lucida drawing of flower, two tepals and stamens removed showing the arrangement of parts (st = stigma). Additional symbols used in all descriptions include bract (B), ad for adaxial, ab for abaxial.


A
AXIS
C
B
D

abaxial portion of the carpel (Fig. 2.1D). Three elongate staminodia occupy the other stamen positions (Fig. 2.1C) . The anther is strongly introrse and the elongate thecae are parallel. The broad filament is adnate to the tepal. There is little anatomical differentiation of the filament and the broad connective that extends distally beyond the microsporangia resulting in an appendage (Venkata Rao, 1967). The carpel suture is adaxial in the median sagittal plane. The carpel is slightly curved abaxially similar to the perigon. The stigmatic papillae differentiate on the sub-distal portion of the suture extending proximally on the abaxial side of the carpel for a short distance (Fig. 2.1C). The flowers and floral organs are glabrous. There is little morphological or anatomical differentiation between the sessile ovary and style. There are numerous, laterally attached, bitegmic, orthotropous ovules in the ovary. The thin-walled fruits are dehiscent along the suture and bear numerous laterally flattened, winged seeds. Four laminar and cuneate nectariferous scales or lobes are present in alternitepalous positions in the perfect flowers (Fig.1C). In male flowers, the nectaries are irregular in shape (Fig. 2.24).

Organogeny - In the axil of a leaf or reduced leaf, the primary inflorescence meristem is inititated. The first floral bracts (pherophylls) are produced 180 degrees apart in frontal positions, succeeded by a subopposite pair in a plane perpendicular to the plane of the first two (Fig. 2.2). Subsequent bracts are initiated in a low spiral. Each bract is cuneate in shape and elongate hairs develop on the distal apex and margins (Fig. 2.3). Within the axil of each bract, a frontally oblate floral meristem develops that will produce a single flower. The floral meristem broadens to approximately 180 by 100 μm and the surface cells appear quite large (Fig. 2.4; Johnson and Briggs, 1975). The first two tepals are initiated subopposite ly from the lateral flanks in the median frontal plane (Fig. 2.5). The third tepal is initiated in an abaxial position followed by the initiation of the adaxial tepal (Fig. 2.6). Stamen primordia are initiated in antetepalous positions in a

Figs. 2.2-17. Early organogenesis and morphogenesis of Placospermum coriaceum flowers. In all images, the floral apices are aligned with the abaxial side of the flower at the bottom of the image except in the oblique images in 14, 17 and adaxial view in 11. **2-3**. Partially dissected inflorescence axes illustrating the anthotactic arrangement of successive bracts. **4**. Floral meristem before tepal initiation. **5.** Initiation of the first two tepals on lateral sides of the floral meristem. **6.** Initiation of the abaxial tepal. **7.** Lateral and abaxial tepals removed showing floral meristem and adaxial tepal. **8.** Oblique side view showing successive stamen initiation; the adaxial stamen has not been initiated. **9.** Tepals removed, showing all four stamen primordia and the remaining floral meristem. **10**. Enlargement of the remaining floral meristem and early carpel transformation. **11.** Adaxial view of the cleft on the adaxial side of the carpel primordium. **12.** Aestivation of the tepals in a functionally male flower; the sides are all valvate but the adaxial and abaxial tepal tips converge; the lateral tepal tips do not come into contact. **13.** Early morphogenesis of the fertile anther; the four microsporangia have been defined by the transverse (tr-arrow) and median furrows (m-arrow). **14.** Adaxial anther and adnate stamen removed from flower showing the extent of adnation between the two structures (brackets) and the short Vorläuferspitze (arrow). **15.** Abaxial perspective of partially dissected flower illustrating the position of the cleft on the abaxial side of the carpel. **16.** Dissected preanthesis bud showing the fertile adaxial anther, the rugose connective, filament and appendicular connective. The anther sacs are strongly introrse. **17.** Oblique frontal view of carpel; the tepals and stamens have been removed, showing the elongating carpel and the margins extending over the summit. **Scale bars** = 250 μm in 2, 3, 17; 50 μm in 4-9; 100 μm in 10-13; and 500 μm in 14-16.



Figs. 2.2-17

similar sequence as the tepals, with the frontal pair first (Fig. 2.7) followed by the successive pair in the sagittal plane (Figs. 2.8, 9). After stamen initiation, the remaining floral meristem is widest in the frontal plane (Fig. 2.9), approximately 100 by 80 μm, and enlarges to approximately 200 by 150 μm and about 90 μm high prior to the first signs of differentiation into a carpel (Fig. 2.10). Adaxial and marginal growth result in the formation of the cleft along the adaxial side of the carpel primordium (Fig. 2.11). The cleft extends to the base of the carpel.

Organ differentiation - *Tepals*- - Aestivation of the tepals is sagittally incomplete valvate. In the perfect flowers, the distal portions of the frontal tepals converge over the inner floral organs. The distal tips of the sagittal tepals do not touch one another but are marginally appressed to the frontal tepals (incomplete-valvate). In the functionally male flowers, the sagittal tepals contact one another but the frontal tepals do not contact one another (Fig. 2.12). It should be noted that this type of incomplete aestivation in the andromonoecious flowers is uncommon among proteaceous taxa examined

The tepals become thick prior to and during aestivation. Each tepal (not the recurved tips of the tepals) begins to heighten (Figs. 2.8, 13) and a spine develops on each tepal (arrow in Fig. 2.13). At anthesis, the spine is approximately three mm in length on the adaxial tepal (Fig. 2.14) but is shorter on the other tepals. The margins of the tepals remain tightly interlocked.

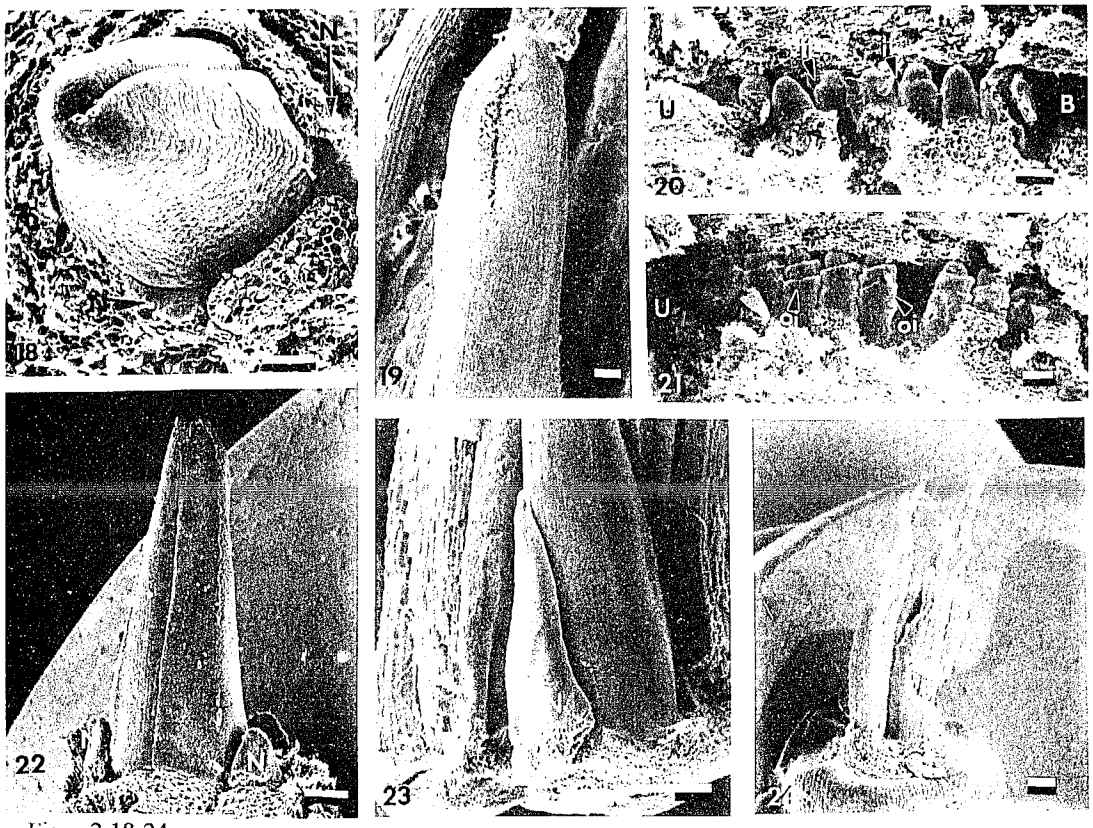
Anthers - On the adaxial anther primordium, a median furrow develops, demarcating the region that will separate the lateral pairs of microsporangia (thecae). A transverse furrow bisecting each side of the young anther defines the microsporangia when the anther primordium is approximately one mm high (Fig. 2.15). The connective appendage and connective base are already distinct (Fig.13). After the earliest stages of thecal formation, intercalary growth begins between the filament and the opposing tepal

member (arrow in Figs. 2.14, 16). The apical portion of the anther elongates and becomes a connective appendage (* in Fig. 2.14). The connective and broad filament expand and become rugose prior to anthesis (compare Figs. 2.14 and 16). The rugose part of the filament extends basally to the floral receptacle. At anthesis, the single fertile stamen dehisces along lateral lines between the thecae and is strongly introrse (Fig. 2.16).

Carpel -The carpel elongates. The margins extend across the top of the carpel (Figs. 2.17, 18, 104A). Later in development, the carpel grows differentially so that the suture (the fused margins) extends shortly down the abaxial side of the carpel as well (sequentially compare Figs. 2.11, 18, 17, 15, 19, 22). It should be noted that the suture extends adaxially from the carpel base to and over the carpel summit (adaxial view in Fig. 2.22; abaxial view in Fig. 2.19; diagrammed in Fig. 2.104A). Along the free margins on the abaxial side of the carpel, elongate stigmatic papillae develop (Fig. 2.19). Ovules are initiated submarginally in an acropetal sequence within the loculus. The ovules enlarge (Fig. 2.20) followed by the initiation of the inner integument. The outer integument forms later, proximal to the inner integument (Fig. 2.21). It should be noted that the order of maturation among the ovules appears to be bidirectional, with the distal and proximal ovules maturing after the middle ovules (Figs. 2.20, 21). In the functionally male flowers, a carpel is initiated but aborts.

Nectaries - Four alternetepalous nectary lobes are initiated (Figs. 2.15, 18) after all organs have been initiated and zonal growth has begun between the stamen and tepal. In the perfect flowers, the nectary lobes first become cuneate (Fig. 2.22). Prior to anthesis, the nectary lobes are tapered and acute (Fig. 2.23). In the functionally male flowers, prior to anthesis, the nectaries are irregular in shape, having multiple apical lobes. In some, there is intercalary growth between and beneath the four lobes resulting in a complete or partial nectarial tube (Fig. 2.24).

Figs. 2.18-24. Morphogenesis of the stigma, ovules and nectaries in Placospermum coriaceum. **18.** Polar view of carpel. Two of the nectaries (arrowhead) have been initiated from the floral receptacle in alternitepalous/alternistaminous positions. **19.** Abaxial view of the carpel showing papillae differentiating on the stigma. **20.** The early development of ovules showing a bidirectional maturation from the middle ovules towards the base(B) and top (U) of the loculus. The inner integument (ii)has been initiated in the middle ovules. **21.** Later stage of ovule development showing the initiation of the outer integument (oi). **22.** Adaxial view of carpel showing the suture that extends to the carpel base as well as the enlarging nectariferous lobes (n). **23.** Frontal view of enlarged laminar nectariferous scale on a perfect flower. **24.** Mature nectaries of a functionally male flower that have multiple apical lobes and a partial nectarial tube. **Scale bars** = 100 μm in 18-21; 250 μm in 22-24.



Figs. 2.18-24

Persooniinae - Two genera are present in subtribe Persooniinae, Garnieria and Persoonia (sensu Weston, 1983). Johnson and Briggs (1975) divided Persoonia (over 30 species) into four genera that were subsequently rejected by Weston (1983). Species of Persoonia grow in tropical and subtropical Australia and New Zealand (Johnson and Briggs, 1975; Weston, 1983; Wrigley and Fagg, 1988). The monotypic Garnieria spathulaefolia Brongn. and Gris. occurs in New Caledonia. Inflorescence morphology differs between the two genera. Garnieria has simple, blastotelic, axillary racemes (Weston, 1983). Most Persoonia species have auxotelic shoots and the flowers are borne in leaf axils in distinct regions along the shoot (Weston, 1983). In many of the taxa with flowering branches (sensu Weston and Johnson, 1991) there are sometimes terminal racemes (Weston and Johnson, 1991). In addition, some taxa have axillary racemes. Floral characters that define Persooniinae include drupaceous fruits, yellowish flowers, and orthotropous ovules (two in Persoonia species and numerous in Garnieria).

Among members of Persooniinae examined and described here (Garnieria spathulaefolia, Persoonia myrtilloides, P. falcata) early flower development is very uniform but later development varies. P. myrtilloides will serve as an example of this type. Two species that were examined but not described, P. oblongata R. Br. and P. mollis R. Br., have a similar ontogeny to P. myrtilloides. Another species, Persoonia falcata, is both morphologically and developmentally diverse, associated with the strongly zygomorphic flowers. Its development will be separately described. Mature character variation among the taxa is illustrated and described.

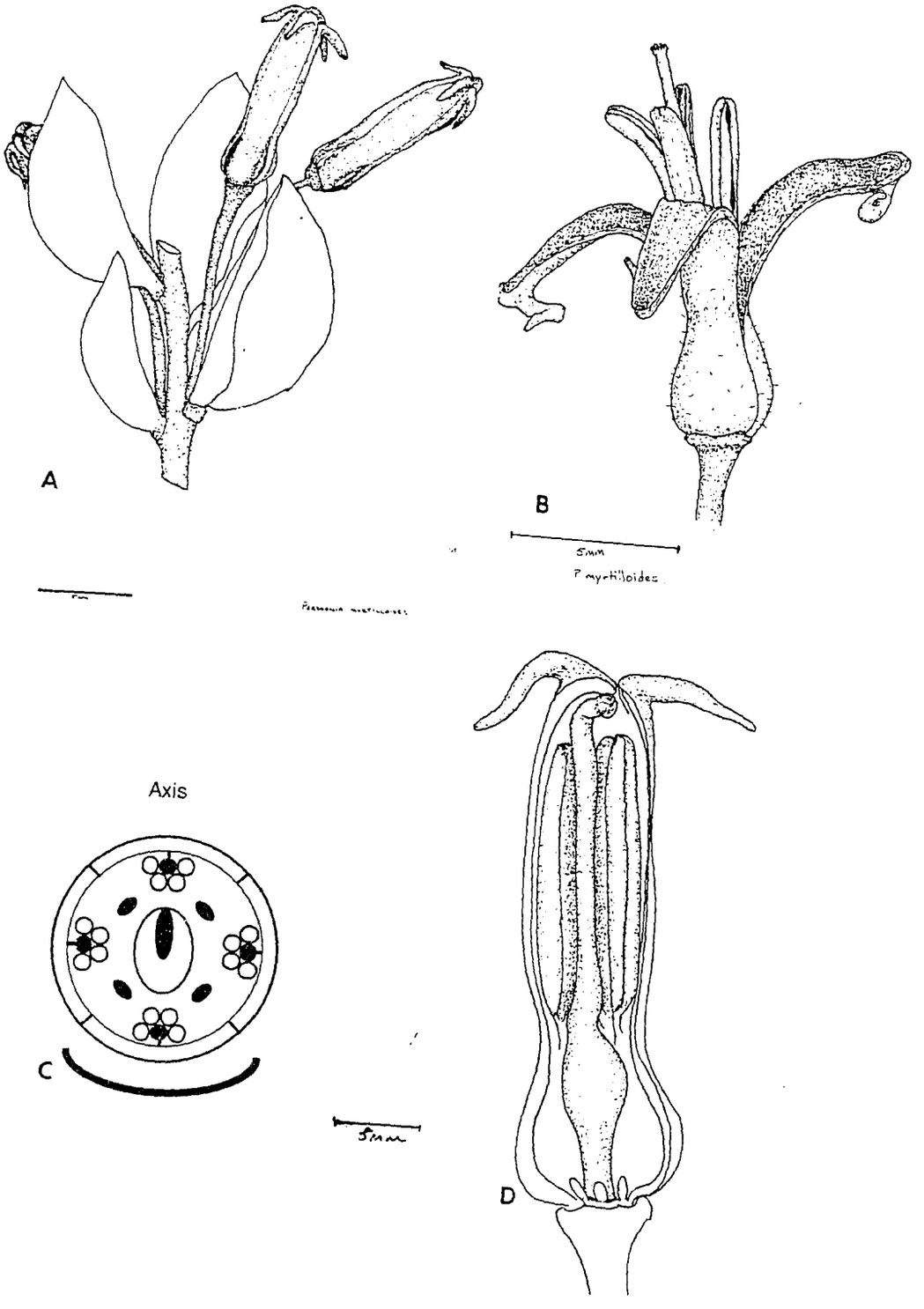
Persoonia myrtilloides - Organography - (Figs. 2.25-53) - Toward the end of an auxotelic shoot, approximately 10-20 yellow flowers develop, each flower in a leaf axil (Fig. 2.25A; Weston, 1983; Weston and Johnson, 1991). There are distinct flowering

regions along the shoot. Such flowering regions have been called 'flowering branches' (Weberling, 1989). Each flower has an elongate reddish, hirsute pedicel.

At anthesis, the yellow tepals reflex by arching away at about the middle of each perianth lobe, distal to the point of anther filament/tepal adnation (Fig. 2.25B). Each tepal tip has an acute elongate spine (Fig. 2.25D). The anthers are white at anthesis and stand erect in the flower, surrounding the erect style. The glabrous carpel has a distally capitate stigma with elongate papillae, a style, a globose ovary containing two submarginally attached orthotropous ovules, an elongate narrow stipe (Fig. 2.25D) and or a disk between the receptacle and stipe. There are four cylindrical, apically rounded nectariferous scales about 2 mm long in alternitepalous positions (Fig. 2.25C-D).

Organogenesis - Within leaf axils along the stem, floral meristems develop (Figs. 2.26, 27). The meristem becomes frontally oblate (Fig. 2.27: approximately 130 x 90 μm) prior to the successive initiation of the first two tepals in the frontal plane (Fig. 2.28). On the adaxial side, the third tepal is initiated (Fig. 2.29) followed by the initiation of the abaxial tepal (Fig. 2.30). The lateral stamens are initiated in the frontal plane (Fig. 2.31A) after the tepals, followed by the sagittal stamens (Fig. 2.32A). Following stamen initiation, the floral apex has a two layered tunica over a corpus (Fig. 2.31B). The two layered tunica persists (Fig. 2.32 B) as the frontally broad (approximately 120 x 90 μm) floral meristem enlarges to about 160 x 140 μm and a height of approximately 90 μm (Figs. 2.33 A-B). During enlargement, periclinal divisions in the inner tunica occur, especially on the abaxial side, and the organization of the subsurface layer of tunica begins to break down (arrows in Fig. 2.33B). The cleft develops on the adaxial side of the carpel (Fig. 2.34A) and does not extend to the carpel base (Fig. 2.35A). The sagittal section (Fig. 2.34B) shows the early stages of the 'chairlike' outline of the carpel primordium (Fig. 2.35B) and the cross zone (querzone) on the lower adaxial side also

Fig. 2.25A-D. Floral and inflorescence diagrams of Persoonia myrtilloides. **A.** Free-hand drawing of flowers on elongate pedicels in leaf axils. **B.** Camera lucida illustration of flower at anthesis, the upper portion of the tepal lobes reflexing away, the lower portions staying fused. On the tip of each tepal is an elongate Vorläuferspitze. The anthers are held erect around the style with its capitate stigma. **C.** Floral diagram showing arrangement of tepals, stamens, carpel and nectary. **D.** Longitudinal camera lucida drawing of flower showing the arrangement of parts; two tepals and stamens have been removed. The carpel is stipitate and on a disk.


A
B
5mm
P myrtilloides
Axis
C
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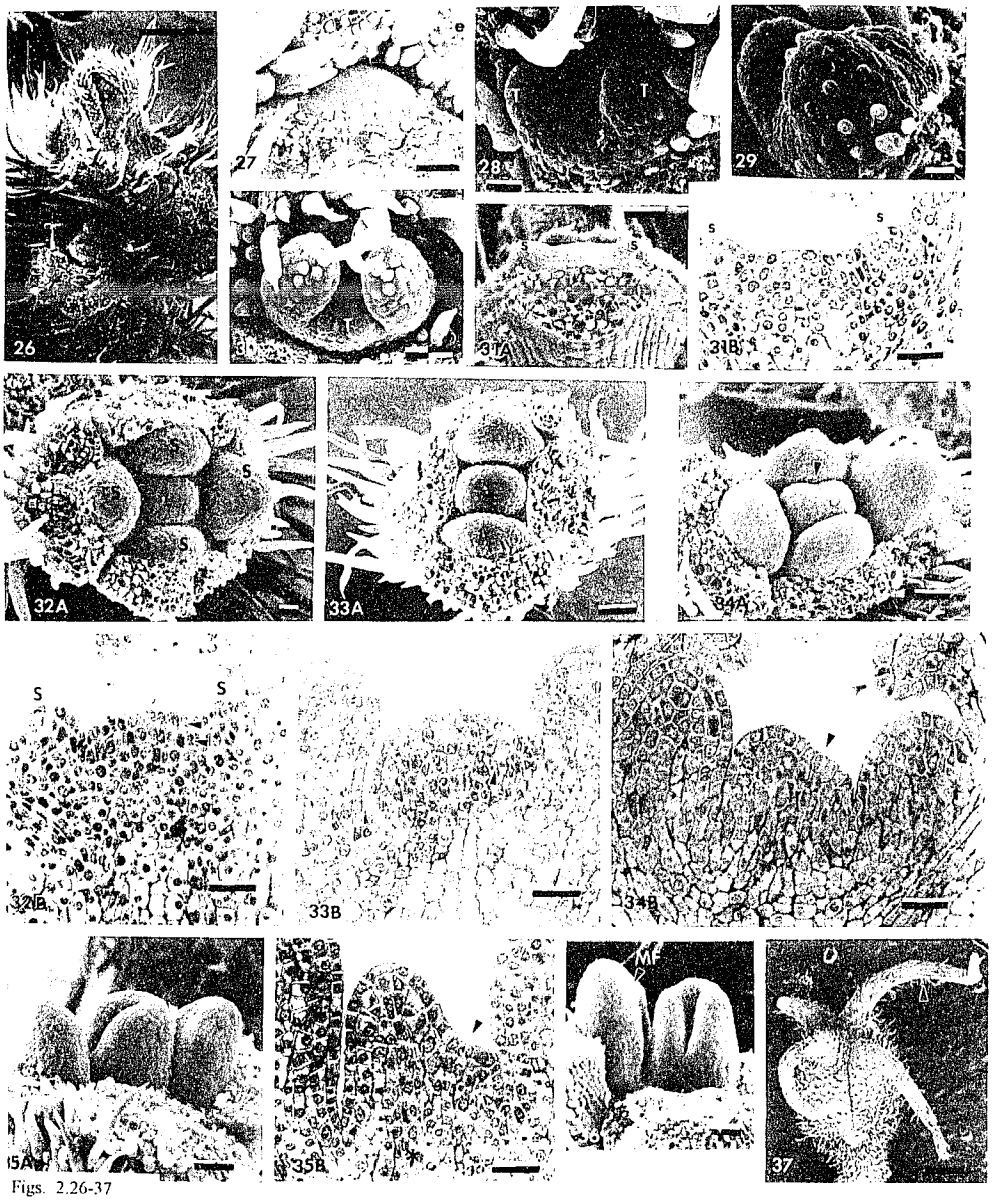
D

Figs. 2.26-37. Early organogenesis of Persoonia myrtilloides flowers. Symbols: MF=median furrow; s=stamen; T=tepal; V= Vorläuferspitze. **26.** Partially dissected shoot showing the arrangement of flowers. **27.** Floral meristem before tepal intiation in leaf axil (leaf removed). **28.** Initiation of the first two tepals in frontal positions. **29.** Side view of young flower, showing the initiation of the adaxial tepal (arrow). Trichomes are differentiating on the abaxial surface of the frontal tepal. **30.** Polar view of young bud showing the initiation of the abaxial tepal. Trichomes are differentiating on both of the frontal tepals. **31A.** Adaxial view showing stamen initiation in frontal positions. **31B.** Longisection of flower at a slightly later stage. The sagittal stamens are present (arrows) and the remaining floral meristem has a two-layered tunica-corpus arrangement. **32A.** Oblique polar view showing the enlargement of the floral meristem. **32B.** Sagittal longisection of flower at a similar stage showing the tunica-corpus arrangement of the meristem. **33A.** Polar view of early carpel primordium. **33B.** Sagittal longisection of flower bud at similar stage showing the break-down of the tunica-corpus arrangement. **34A.** Oblique polar view of cleft development on adaxial side of flower. **34B.** Sagittal longisection of flower at similar stage showing the early signs of a cross zone (arrow). **35A.** Side view of young carpel primordium; the frontal anthers and all four tepals have been excised. The carpel cleft does not extend to the carpel base. **35B.** Sagittal section showing the chairlike outline of the carpel primordium, and the cross-zone (arrow) on the adaxial side. **36.** Adaxial view of slightly later carpel stage. The tepals and three stamen primordia have been removed. The median furrow has formed on the stamen primordium. **37.** Oblique polar view of floral bud. Tepals show sagittally incomplete aestivation; the frontal tepal tips are appressed to one another and the sagittal tepal tips do not touch one another but fit between the laterals. An elongate spine-like process or Vorläuferspitze is present on each tepal lobe. **Scale bars** = 50 μm in 27-36; 500 μm in 26 and 37.



Figs. 2.26-37

described as a "bowl-shaped primordium" (Sampson and Kaplan, 1970) tilted towards the adaxial side. The entire floral apex is converted into the carpel. No floral apex residuum persists surrounding the carpel base (Fig. 2.34B and 35B). The tunica-corpus organization deteriorates prior to the development of the cleft (Figs. 2.33B and 34B).

Organ Differentiation - *Tepals* - The frontal tepal tips converge above the other floral organs and come into contact with one another. The sagittal tepals do not contact one another but fit between the frontals (incomplete aestivation-Fig. 2.37). Trichomes develop on the outer surface of each tepal (Fig. 2.30). It should be noted that on each tepal the differentiation of trichomes occurs sequentially among tepals. The trichomes develop on the frontal tepals first, followed by the successive differentiation of trichomes on the sagittal tepals (compare Figs. 2.26, 30). After aestivation is completed, the tepals each produce a spine-like process from the abaxial side of the tepal (Fig. 2.37). The structure resembles a Vorläuferspitze found in leaves of some unifacial monocot leaves (Kaplan, 1973) and the sepals of various angiosperms (Baum, 1951; Baum-Leinfellner, 1953; translated in Weberling, 1989 by Pankhurst as an excurrent unifacial tip). That is, it is the product of the abaxial portion of the tepal and apparently results from reduced growth of the actual tip (Kaplan, 1973). At anthesis, the tepallary outgrowth (Fig. 2.25B,D) is approximately three mm long. Trichomes continue to develop on the outer (abaxial) surface of the tepal. Prior to anthesis, the adaxial epidermal cells of the tepal become papillate (Fig. 2.38) next to the epitepalous stamen filament. The epidermal cells of the adnate filament have similar papillae, distributed diffusely. The basal one-third of each tepal becomes wider than the upper portion (Figs. 2.25B, 46) prior to anthesis.

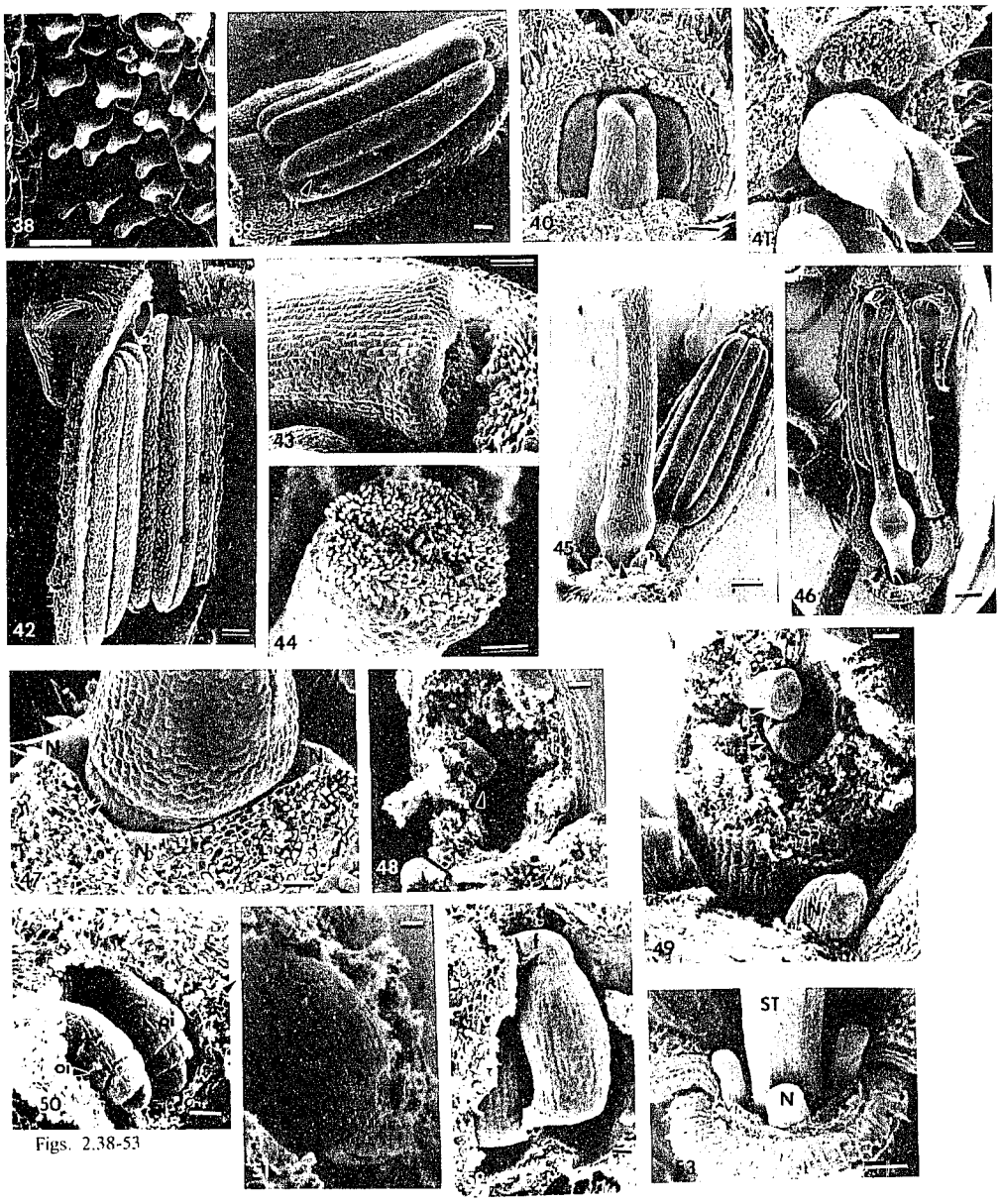
Stamens - At a stamen height of about 200 μm, the median furrow forms on each of the stamen primordia (Fig. 2.36). Demarcation of the transverse furrows

occurs later (arrowhead, Fig. 2.39). There is no appendicular connective present distal to the thecae (Figs. 2.39, 45). Concomitant with early anther development, zonal growth beneath and between the base of the anther and tepal results in an epitepalous condition (Figs. 2.40, 41, 46). The adaxial surface of the filament remains distinct although fused to the tepal on the other side. Flask shaped papillae differentiate on the filament. At anthesis, there is no appendicular connective present, and the lateral dehiscence lines extend to the filament and over the top of each thecal pair (Fig. 2.42).

Carpel - As the carpel enlarges, the suture extends along the adaxial side of the carpel midway over the summit (Fig. 2.41). On the adaxial side, the suture does not extend to the carpel base (Fig. 2.40). When the carpel is approximately one mm in height, continued growth of the distal portion of the margins results in the suture extending from the adaxial side over the top of the carpel although it does not extend to the abaxial side (Fig. 2.41). The distal portion of the carpel begins to expand centripetally (Fig. 2.43). Elongate papillae differentiate on the capitate stigma (Fig. 2.44). The style is elongate (Fig. 2.45). When the carpel is approximately 500 μm in height, a disk becomes demarcated between the carpel and receptacle (Fig. 2.47). Following the demarcation of the disk, a stipe develops between the disk and glabrous ovary (Figs. 2.45, 46). The disk is persistent (Fig. 2.25D).

Two ovules are initiated submarginally in the upper portion of the locule (Fig. 2.48). The two ovule primordia enlarge, the inner integument developing prior to the outer integument (Figs. 2.49, 50). The ovule funiculus elongates as the outer integument extends beyond the micropylar end of the young ovule (Fig. 2.51). Crowding and ovule enlargement force the ovules toward the base of the ovary although it should be noted that the ovules are orthotropous with an elongate funiculus (Fig. 2.51). In addition, the growth of the outer integuments produces a bent tubular micropylar canal that faces the wall of the locule (Fig. 2.52).

Figs. 2.38-53. Morphogenetic stages of flower development in Persoonia myrtilloides. **38.** Papillate cells of the adaxial epidermis of the tepals. **39.** Adaxial view of young stamen. The four microsporangia have been defined by the transverse furrows (**tr-arrowhead**). Adnation between the tepal and filament is already present. **40.** Adaxial view of partially dissected bud showing the early adnation of the stamen primordium to the tepal (*). The carpel cleft extends along the adaxial side to the summit but not to the carpel base. **41.** Oblique polar view of carpel showing the adnation zone between the excised stamen primordium and the tepal on the adaxial side (*). The carpel suture extends partway across the summit of the carpel. There is enlargement of spaces in alternetepalous positions where the nectariferous lobes will develop (arrow). **42.** Two anthers and their opposing tepal members prior to anthesis. No appendicular connective is present. The lateral dehiscence lines extend from the filament and over the top of each thecal pair. **43** Enlargement of the distal portion of carpel. **44.** Elongate papillae on the capitate stigma. **45.** Side view of carpel showing an elongate style (st), ovary and the early development of the stipe (arrow). Two nectariferous lobe primordia are visible (**N**). **46.** Older flower showing stipe, glabrous ovary, elongate style and stigma, slightly compressed in bud. **47.** Oblique adaxial view of receptacle and carpel at approximately 500 μm height. The disk between the ovary and receptacle has become demarcated. Four nectariferous lobes develop in alterni-tepalous positions (only three visible). **48.** Dissected early ovary showing the initiation of two ovules in the upper portion of the locule. **49.** The inner integument developing on the ovules. Enlarged nectaries surround the carpel; the stipe is just beginning to elongate. **50.** The outer integument has developed on the two ovules. **51.** In older ovule, the outer integument envelops the inner integument forming the micropyle. The funiculus is becoming elongate. **52.** Ovule at anthesis. The funiculus is elongate, and the ovule points towards the base of the locule. Note the bent micropylar canal. **53.** Floral receptacle at anthesis showing the mature nectariferous lobes surrounding the stipe. **Scale bars:** 38 = 50μm; 39-41, 43, 44 = 100μm; 42, 45, 53 = 250μm; 46 = 500 μm; 47-52 = 50μm.



Figs. 2.38-53

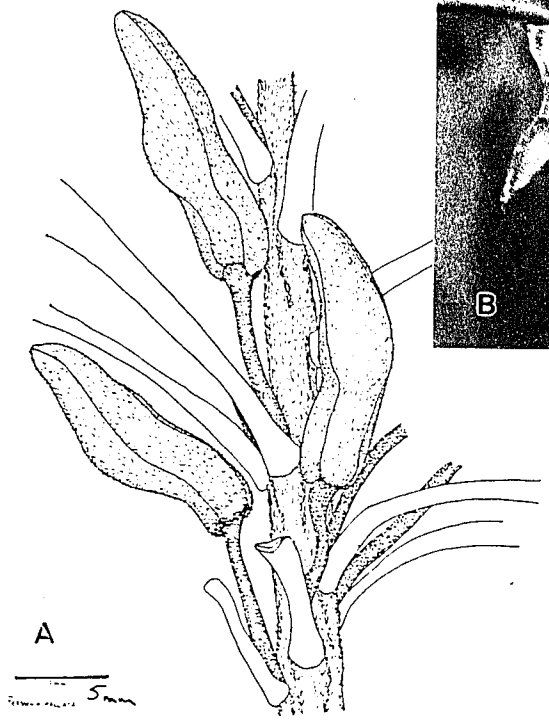
Nectaries - Four alternitepalous nectary lobes are initiated (Fig. 2.41, 47) after zonal growth between the anthers and tepals has begun and after after demarcation of the carpellary cushion or disk (compare Figs. 2.41 and 47). The nectary lobes enlarge (Fig. 2.49) becoming approximately 500 μm high at anthesis (Fig. 2.53).

Persoonia falcata R. Br. - Organography (Figs. 2.54-73) - Flowers are borne singly in leaf axils (sometimes cuneate bracts or pherophylls) along an auxotelic shoot (Fig. 2.54A, 54C, 55) (Weston, 1983). Along the indeterminate shoot, there are distinct regions: a flowering region composed of 10-20 flowers, each flower borne in a leaf axil, and a vegetative region that is composed of nodes, internodes and 10-20 dormant vegetative buds, each one borne in a leaf axil. Morphologically, the internodes in the flowering region tend to be shorter than the internodes in the vegetative bud region.

Each flower is pedicellate (Fig. 2.54A) and strongly zygomorphic in three ways; the curvature of the perigon, reflexion of the tepals at anthesis and carpel form (Fig. 2.54B, D). In addition, 35% of the flowers from three different individuals (five flowering branches sampled from each plant) in a population were five-merous.

Organogenesis - A single floral meristem develops in each of several leaf axils on the stem (Fig. 2.55). From a frontally oblate floral meristem, the tepals are initiated sequentially: a frontal tepal first (Fig. 2.56), followed by the opposing frontal tepal (Fig. 2.57). The third tepal is initiated adaxially followed by the initiation of the abaxial tepal (Fig. 2.58). In some flowers, a fifth tepal is initiated and is borne between the first tepal initiated and the adaxial tepal (in a 2/5 or 1/5 phyllotaxis; Fig. 2.59). Stamen primordia are initiated in a similar sequence (not illustrated). In five-merous flowers, a fifth stamen is initiated opposite the fifth tepal. Directly after stamen initiation, the remaining floral meristem is convex and frontally broad (~120 x 80 μm; Fig. 2.60). As

Figs. 2.54A-D. Floral and inflorescence diagrams of Persoonia falcata. **A.** Flowers on long pedicels in leaf axils. **B.** Flowers at anthesis are zygomorphic, the appendicular connective of each anther is exserted. **C.** Floral diagram showing arrangement of tepals, stamens, carpel and nectaries. **D.** Longitudinal camera-lucida drawing of flower showing the arrangement of parts; two tepals and and anthers have been removed. The carpel is shortly stipitate and has a disk at the base (in black). The adaxial side of the flower is marked with an asterisk.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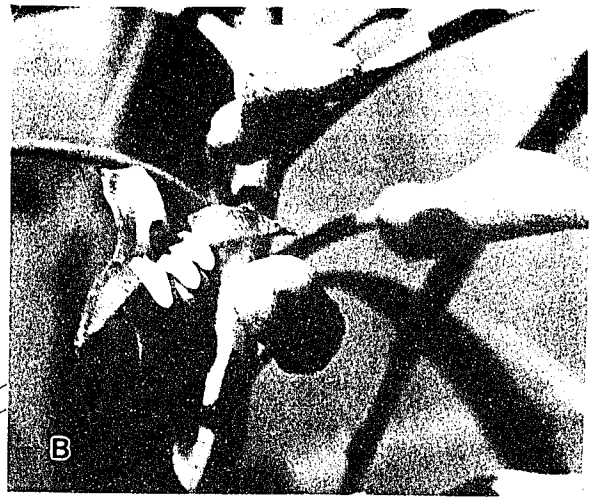
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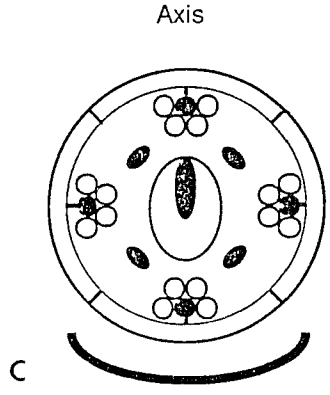
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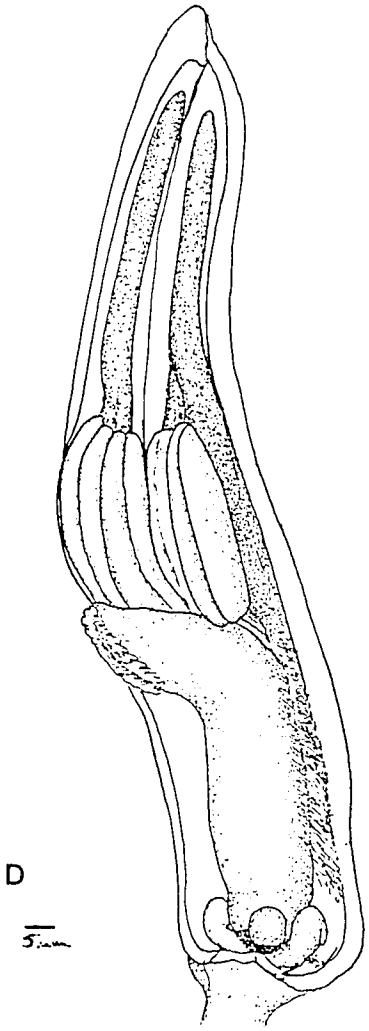
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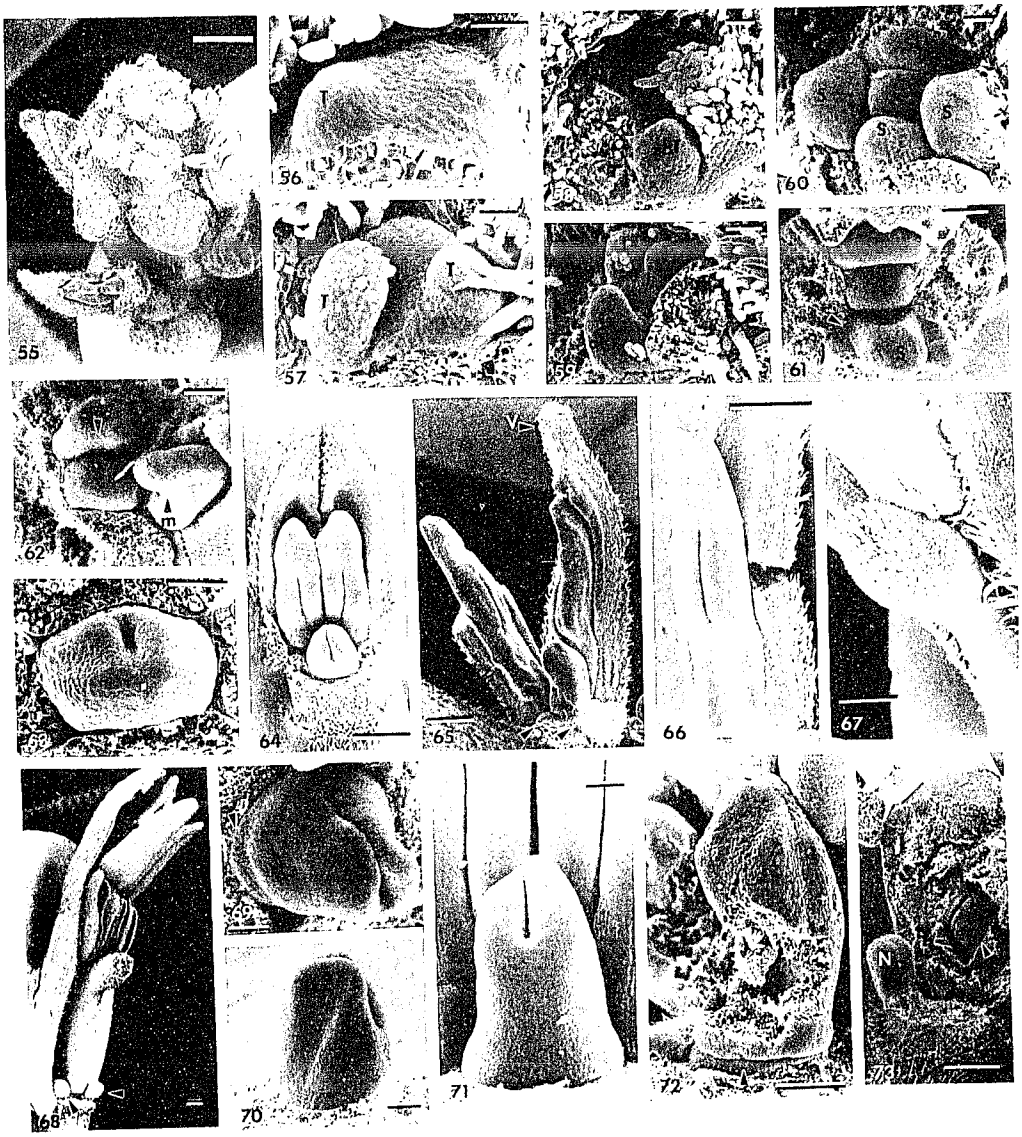
Figs. 2.55-73. Floral organogenesis of Persoonia falcata. **55.** Partially dissected shoot showing the arrangement of flowers on the flowering branch. **56.** Floral meristem in leaf axil, the first tepal (**T**) in a frontal position is present. **57.** The initiation of the second tepal in a frontal position of the floral meristem. Trichomes are differentiating on the abaxial surface of the first tepal. **58.** The initiation of the sagittal tepals in a four-merous flower. The frontal tepal has been removed. **59.** The initiation of a fifth tepal (**T5**) primordium in a position between the adaxial sagittal and one of the frontal tepals. **60.** All four stamens in a four-merous flower are present and the floral meristem is enlarging in the center of the flower. **61.** Increased enlargement of the floral meristem. The abaxial stamen has become adnate to the tepal (arrow). **62.** The carpel primordium has a cleft. The median furrow of the anthers is present. **63.** Polar view of carpel primordium, with the cleft on the adaxial side and not extending to the carpel base. **64.** Polar view of five-merous flower, the abaxial and frontal tepals and anthers removed. The cleft extends across the summitt of the carpel to the abaxial side. The transverse furrows of the anthers are becoming evident. **65.** Side view of partially dissected flower showing the short Vorläuferspitze on the tepal (**V**, arrow). The cleft on the adaxial side of the short carpel does not extend to the carpel base. The nectaries have been initiated (arrows). **66.** Longitudinal view of anther showing the epitepalous filament, the developing appendicular connective, the four young thecae and papillae developing on the adaxial surface of the tepal next to the adnate filament. **67.** Stigma before anthesis showing the abaxially curved style and capitate stigma. **68.** Longitudinal view of pre-anthesis flower showing the nectaries (arrows), the abaxially curved style of the carpel, the dehiscence of the anthers and the appendicular connectives. **69.** Enlargement of the carpel, showing the cleft extending along the adaxial side of the carpel (not to the carpel base) and over the summit. The disk (arrows) has become demarcated. **70.** Side view of comparable carpel stage showing the cleft. **71.** Abaxial longitudinal view of carpel showing the cleft extending over the summit and down the abaxial side for a short distance. **72.** Side view of carpel showing disk (arrow) and the initiation of the ovule in the loculus. **73.** Enlarged ovule (arrow) and one of the nectaries. **Scale bars**: 55 = 1mm; 56-60=50μm; 61-63 and 69-71=100μm; 64-68=500μm; 72=250μm.



Figs. 2.55-2.73

the remaining floral apex converts to a carpel, it enlarges to approximately 220 x 180 μm (Fig. 2.61) and is approximately 160 μm high prior to the differentiation of a cleft. The cleft forms as a dimple on the distal adaxial portion of the carpel primordium and does not extend to the receptacle (Fig. 2.62, 63).

Organ differentiation - *Tepals* - Trichomes develop abaxially on each tepal (compare Figs. 2.57, 58). It should be noted that trichomes differentiate sequentially among tepals; the trichomes develop on the frontal tepals first followed by those on the sagittal tepals. Trichomes continue to develop on the abaxial surface of the tepal. The frontal tepal tips converge above the other floral organs and come into contact with one another. The sagittal tepals do not contact one another but fit into the spaces between the frontals. Thus, the tepals have a sagittally incomplete valvate pattern of aestivation. The abaxial portion of the tepal (not the incurved tips) extends resulting in a short spine (Fig. 2.64, 65). Prior to anthesis, the adaxial epidermal cells of the tepals, next to the epitepalous filament, become papillate and continue to elongate (Fig. 2.67).

Stamens - Zonal growth beneath and between the base of the anther and tepal results in a relatively precocious epitepalous condition (arrow in Fig. 2.61). The median anther furrow forms (arrow in Fig. 2.62) at a stamen height of approximately 200 μm followed by the formation of the transverse furrows (arrows in Figs. 2.64, 66). There is an elongate, laminar appendicular connective present distal to the thecae (Figs. 2.54D, 66). The adaxial surface of the filament remains distinct although fused to the tepal (Fig. 2.66). Elongate hairs differentiate on the filament. At anthesis, the laminar appendicular connective is elongate and is exserted from the mature flower (Fig. 2.54B), and the anthers dehisce via lateral lines (Fig. 2.68).

Carpel - The carpel margins expand resulting in the cleft extending across the top of the carpel and down the abaxial side (Figs. 2.69, 70, 71). The cleft extends the

entire length of the adaxial side of the carpel although it does not extend to the carpel base (Figs. 2.65, 69). On the stigma, the suture spreads slightly on the abaxial side: elongate papillae differentiate resulting in a stigmatic area (Fig. 2.67). As the stigmatic area is differentiating, unequal growth of the distal portion of the style continues resulting in an angled or abaxially reflexed stigma (Figs. 2.68, 72; diagrammed in Fig, 104B). When the carpel is about 500 μm in height, a disk or cushion forms at the base of the glabrous ovary (Figs. 2.69, 70, 72). No stipe develops. Two ovules are initiated in the upper part of the loculus (Fig. 2.72). At maturity, the orthotropous ovules have a short funiculus, and the outer integument covers the inner integument (Fig. 2.73).

Nectaries - Four nectariferous lobes are initiated on the floral receptacle in alternitepalous positions (Figs. 2.64, 65, arrows). Five lobes are initiated in the five-merous flowers). The nectary lobes become cylindrical (Fig. 2.73) and at maturity are approximately 500 μm in height (Fig. 2.68).

Garnieria spathulifolia (Figs. 2.74-91) - Simple blastotelic racemose inflorescences develop in leaf axils (Weston, 1983). Ten to sixteen flowers comprese each inflorescence, and each flower is initiated in the axil of a cuneate bract (Fig. 2.74A). Each flower has a short, densely hirsute pedicel (Fig. 2.74B). The four tepals each reflex about forty degrees from the vertical at anthesis (Weston, 1983). Each tepal has an acute, sub-terminal, abaxial spine or Vorläuferspitze (Fig. 2.74B). Each stamen has a broad filament that is partially free and partially adnate to the tepal. The anther is circumferentially latrorse to slightly introrse, having longitudinal dehiscence between the pairs of parallel thecae. No connective appendage extends distally beyond the thecae. The carpel has a terminal, capitate papillate stigma, a straight style, and a glabrous, slightly broadened ovary containing numerous, laterally attached hemitropous ovules. The base of the ovary tapers to a thick short stipe subtended by a disk or

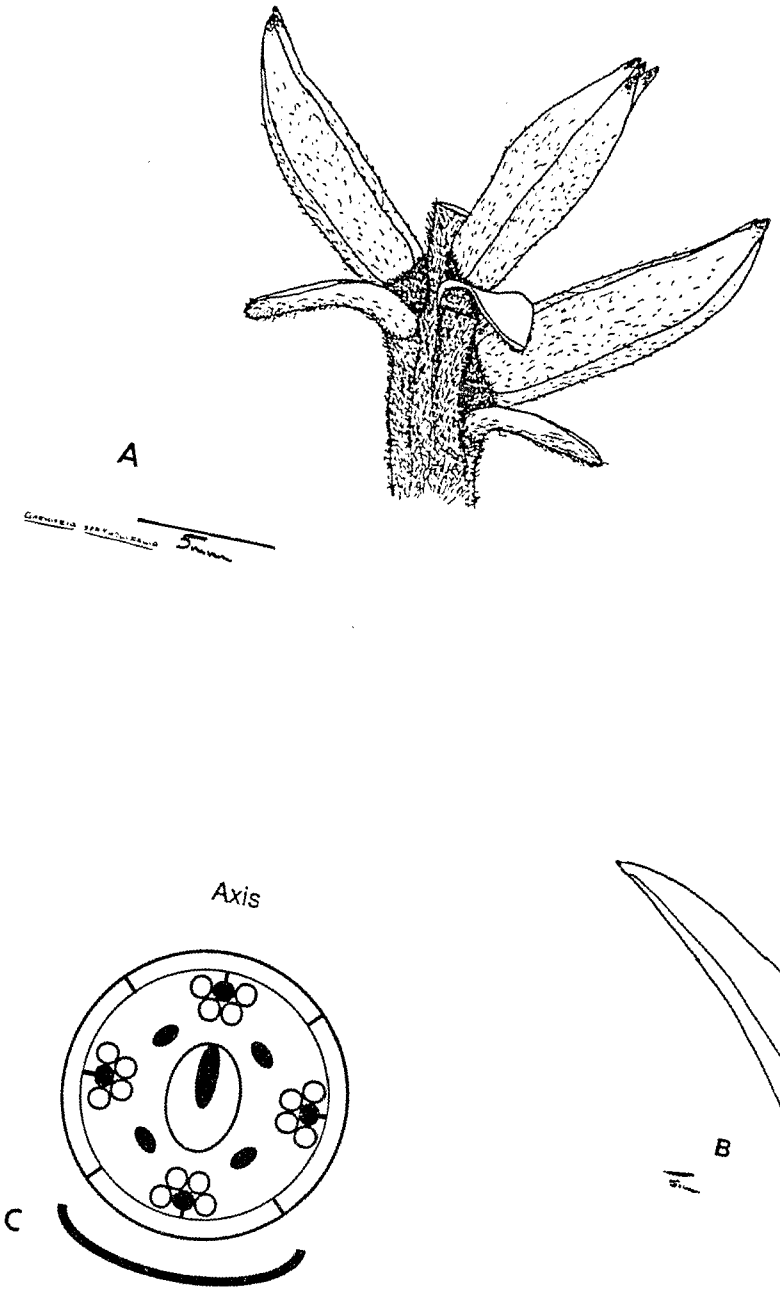
cushion. Each flower has four short, cylindrical, apically rounded nectariferous scales (Fig. 2.74B-C).

Organogeny - A single floral meristem is initiated within the axils of cuneate, fimbriate bracts. The first two tepals are initiated successively and laterally, followed by the successive initiation of the adaxial (Fig. 2.75) and abaxial tepals. The stamens are initiated in a similar sequence, the frontals followed by the sagittals (Fig. 2.76). After stamen initiation, the frontally broad remaining floral meristem (about 120 x 80 μm) enlarges (to approximately 130 μm and approximately 200 μm wide by 150 μm broad) (Figs. 2.77, 78). The cleft forms adaxially and does not extend to the base of the carpel (Figs. 2.79, 80). The pattern of aestivation is sagitally-incomplete-valvate, the frontal tepals come into contact with one another but the sagittal tepals do not contact one another; rather they fit into the corners between the frontals.

Organ differentiation - *Tepals* - At aestivation, the tepal tips incurve. The tepals are relatively thick and the epidermal cells of the valvate margins become interlocked. Prior to anthesis, the inner surface of the tepals becomes rugose and fleshy (Fig. 2.84). Each tepal produces an acutely tapered abaxial spine or Vorläuferspitze (compare Figs. 2.82, 83, 84).

Stamens - Morphogenesis of the stamens begins with the appearance of the median furrow (Fig. 2.80) followed by the appearance of the tranverse furrow (Figs. 2.81, 82). The enlarging thecae overtop the small connective appendage as development continues . At anthesis, the lateral dehiscence lines extend to the filament and over the top of each thecal pair (Fig. 2.85). Intercalary growth begins between and beneath each young stamen and tepal resulting in an epitepalous condition (Figs. 2.82, 84). It should be noted that before adnation of a stamen to each tepal, one can remove the tepals during dissection without affecting the stamen primordia (Figs. 2.78, 80). After adnation

Fig. 2.74A-C. Flower and inflorescence of Garnieria spathulifolia. **A.** Flowers arranged on an inflorescence axis. Each flower is subtended by a cuneate bract and is attached to a short hirsute pedicel. **B.** Longitudinal camera-lucida drawing of pre-anthesis flower. Two tepals and stamens have been removed. On each tepal, there is a Vorläuferspitze (**V**). The carpel is shortly stipitate on a disk. Four nectariferous lobes surround the carpel. The flower has a short, densely hirsute pedicel. **C.** Floral diagram.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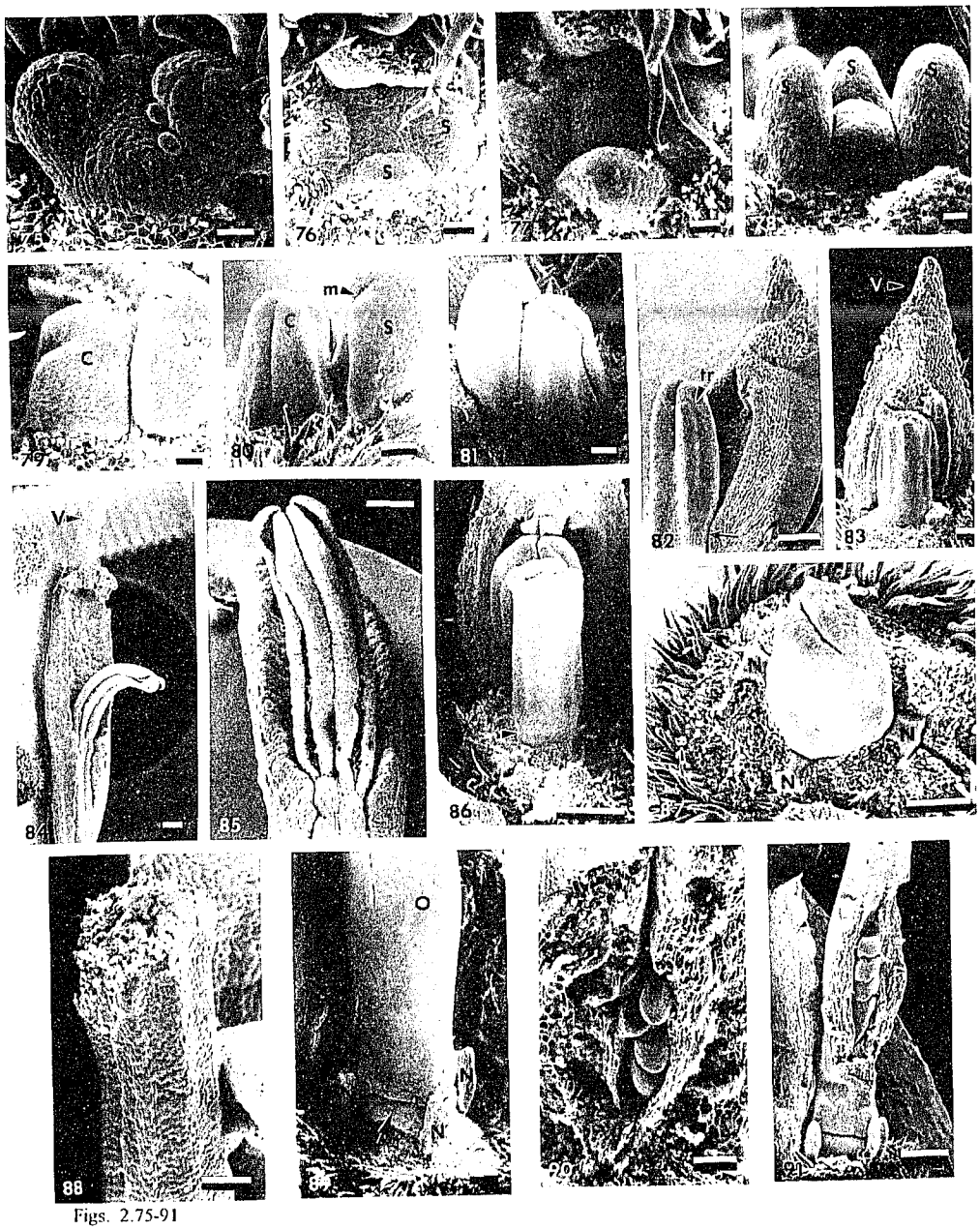
Axis
C
B

Figs. 2.75-91. Floral organogenesis of Garnieria spathulifolia. Symbols: **T** = tepals; **s** = stamens; **C** = carpel; **O** = ovary; **m** = median furrow; **tr** = transverse furrow; **V** = Vorläuferspitze; **N** = nectary. **75.** Floral meristem. The tepals in the frontal plane and the tepal in the adaxial position have been initiated. **76.** All four stamen primordia are present and the frontal stamens are larger than the sagittal stamen primordia. **77.** Enlargement of the floral meristem between the stamens. **78.** Frontal view of the enlarging carpel primordium. Three stamen primordia are present, the fourth one has been removed. **79.** Oblique frontal view of carpel primordium. The cleft has developed on the adaxial side. **80.** Adaxial view of carpel primordium showing the cleft along the adaxial side but not extending to the carpel base. The median furrow has developed on the stamen primordium. **81.** Later stage of carpel development showing the cleft extending over the summit of the carpel. The stamen filament is adnate to the tepal. **82.** Tepal and stamen separated from flower showing the Vorläuferspitze. The transverse furrow has become evident in the young anther. **83.** Frontal view of enlarging carpel and the tepal. The Vorläuferspitze is elongating (arrowhead). **84.** Mature anther adnate to tepal. An acute Vorläuferspitze is present on the tepal. The anthers dehisce via longitudinal slits. **85.** Adaxial view of mature anther showing the longitudinal dehiscence pattern. **86.** Oblique frontal view of enlarging carpel. The disk has begun to form at the base of the carpel (arrowhead) and the top of the carpel has begun to enlarge circumferentially. The nectariferous lobes have been initiated in alternitepalous sites. **87.** Polar view of carpel: The anthers and tepals have been removed. The cleft extends across the summit of the carpel. Four nectariferous glands are developing in alternitepalous sites. **88.** Mature capitate and papillate stigma. **89.** Mature carpel base showing the nectaries, the disk (arrow), the short stipe and the broadened ovary. **90.** Abaxial side of young ovary cut open, to show some of the numerous ovules borne submarginally. **91.** Mature ovary dissected to show the hemitropous orientation of the numerous ovules. **Scale bars**: 74-78 = 50 μm; 79, 80, 89 = 100 μm; 81, 82, 86 = 250 μm; 83-85, 87-88, 90 = 500 μm.



Figs. 2.75-91

between each stamen and tepal, removal of a tepal pulls the stamen off as well (the base of the tepal still present in Fig. 2.81, the tepal/stamen pulled off in Fig. 2.82). The adaxial surface of the filament remains distinct although fused to the tepal abaxially.

Carpel - The carpel becomes elongate, the cleft extending adaxially (but not to the carpel base) and halfway over the top (Fig. 2.81, 86, 87). When the carpel is approximately one mm in height, the carpel tip begins to expand, resulting in the formation of the stigma (Figs. 2.83, 86). Elongate papillae differentiate on the stigma and it appears capitate (Fig. 2.88). The style is elongate and cylindrical (Fig. 2.74B) above the slightly enlarged, glabrous ovary (Fig. 2.91). When the carpel is approximately one mm in height, a disk or cushion becomes demarcated between the carpel and receptacle (Figs. 2.86, 89). Intercalary growth between the cushion and ovary results in a tapered carpel base (Fig. 2.89). Numerous ovules are initiated close to the margins (Fig. 2.90). At maturity, the micropyles of the orthotropous to slightly hemitropous ovules face the base of the locule (Fig. 2.91).

Nectaries - Four lobes are initiated in alternitepalous positions (Figs. 2.86, 87) as intercalary growth begins between and beneath the stamens and tepals. The cylindrical nectary lobes develop to a height of approximately 1 mm at anthesis (Figs. 2.89, 91, 74C).

Bellendeneae. Bellendena montana - (Figs. 2.92-103) - Bellendena montana is a monotypic, small, slow-growing shrub in montane Tasmania. At maturity, the relative growth unit is anauxotelic, the simple, condensed raceme or reproductive axis (Fig. 2.92A) being the terminal product of the shoot meristem. There is a serial transition of the lobed leaves on the vegetative axis to smaller lobed leaves basal to the inflorescence, to cuneate bracts basal to and ascending the base of the inflorescence axis (Fig. 2.92A). Twenty to thirty flowers on diffusely hirsute pedicels are borne on each inflorescence

(Fig. 2.92A). The flowers appear to lack individual floral bracts at anthesis (Venkata Rao, 1971; Johnson and Briggs, 1963, 1975) but they are present earlier.

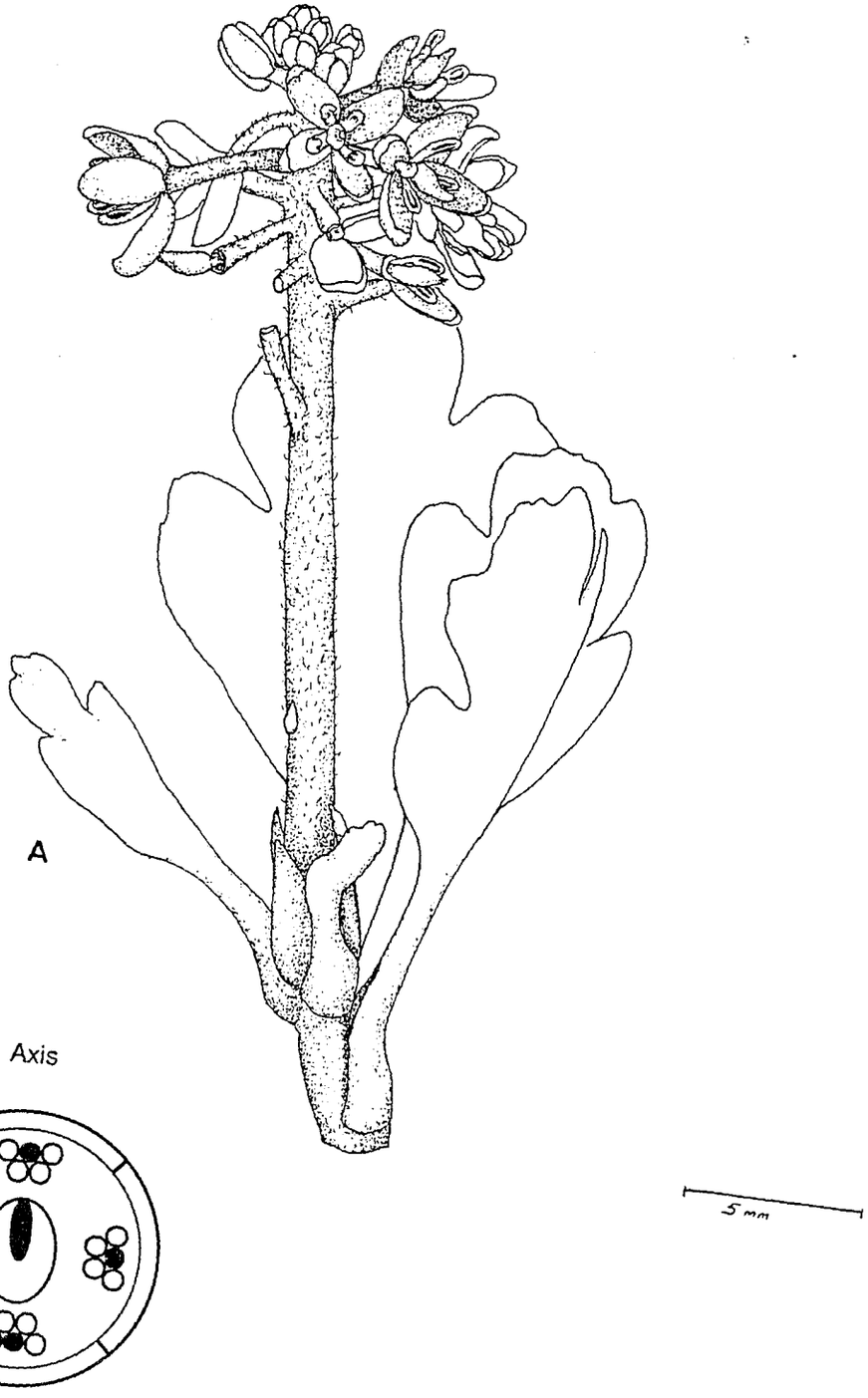
The flowers are creamy white at anthesis, and the glabrous, petalloid tepals remain relatively straight but flair outward as the flower opens (Fig. 2.93). The four anthers curve around the ovary and the basal portion of the style. The anthers dehisce slightly introrsely along longitudinal lines. The filaments are free and not attached to the tepals (Fig. 2.92B). The filament and connective are broad. The thecae are parallel to one another. The gynoecium includes a glabrous, slightly stipitate ovary, a short style with a persistent suture line adaxially (Fig. 2.100), and a punctiform, papillate stigma (Fig. 2.102). Two submarginally attached orthotropous ovules are in the ovary (Fig. 2.103).

Stages of organogenesis were only available from rehydrated herbarium material. The only stages captured from such material show the young stamen primordia and the floral apex (Figs. 2.94, 95) prior to and at formation of the cleft. The cleft of the gynoecium forms adaxially in the sagittal plane.

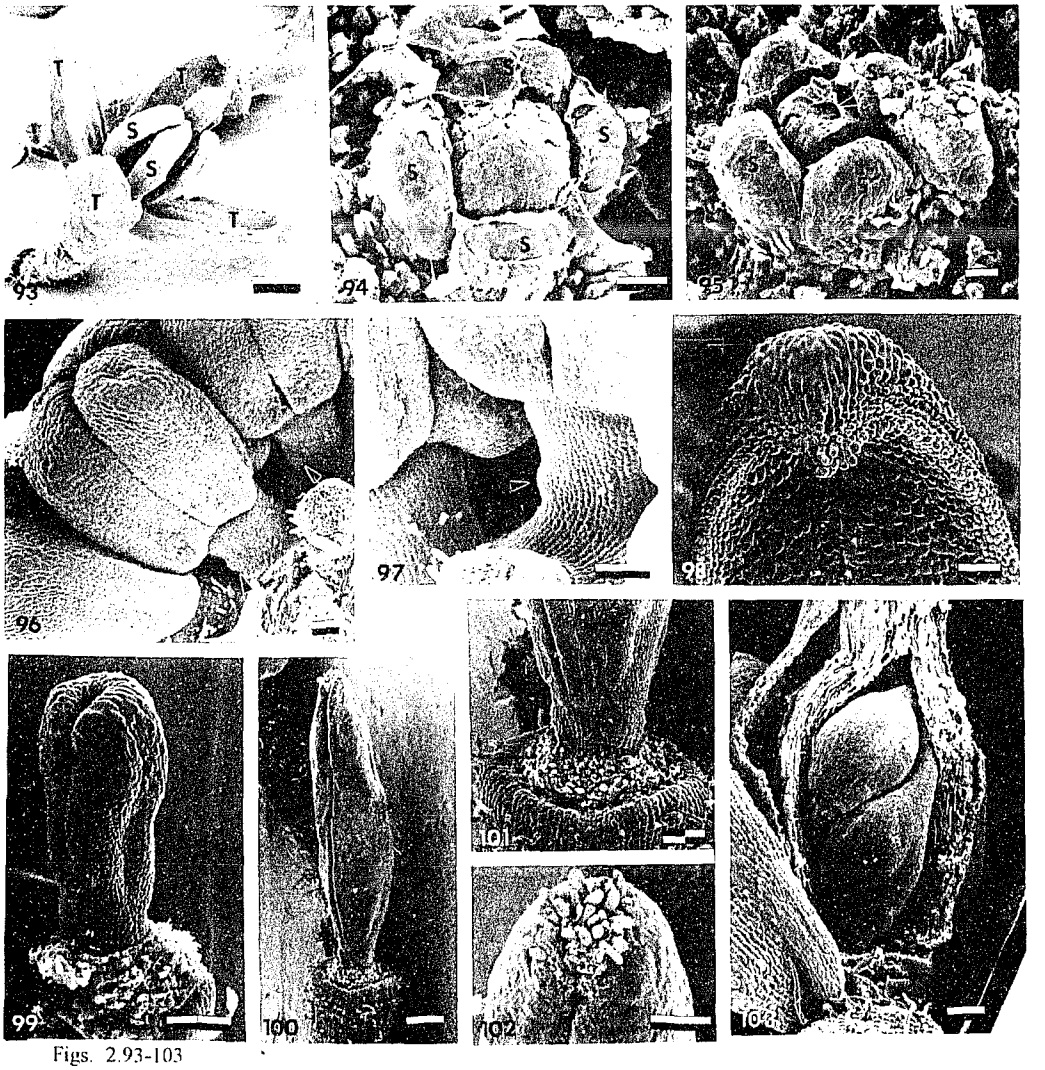
Later developmental stages found in fixed material show rudiments of subtending bracts (Figs. 2.96, 97). These bracts appear to be suppressed following their initiation. After aestivation, in 80% of the flowers, the tips of the frontal tepals become appressed and the sagittal tepals do not contact one another (not shown). In approximately 20% of the flowers on any inflorescence, aestivation is by sagittal tepal contact, not frontal tepal contact. The tepals are glabrous and bear stomata on the abaxial surface (not shown). The distal incurved tepal tips become elongate in bud and the arcuate tip of the tepal (Fig. 2.98) is thicker than the proximal portions of the tepal.

Late stages of carpel development show that the suture extends down on the adaxial side but does not extend to the base of the carpel (Fig. 2.99, 100). The suture

Fig. 2.92A-B. Inflorescence and flowers of Bellendena montana. **A.** Inflorescence terminates the growth of the vegetative axis. There is a serial transformation acropetally up the axis from lobed leaves, to small lobed leaves, to cuneate ensheathing bracts and small tapered bracts on the peduncle. The inflorescence is a condensed raceme with little elongation of internodes. The flowers are pedicellate and at anthesis, the tepals reflex. **B.** Floral diagram showing no adnation between the anthers and the tepals, and no alternitepalous glands around the carpel.


A
Axis
B
5 mm

Figs. 2.93-103. Organogenesis of flowers of Bellendena montana. Symbols: **T**= tepals; **s** = stamens. **93.** Mature flower at anthesis. The four free stamens surround the style and ovary of the carpel, and the tepals are flaring. **94**. Early carpel primordium with young stamens; the tepals have been removed. **95**. Oblique view of carpel primordiumwith the cleft becoming apparent on the adaxial side of the flower. **96, 97.** Side view of young flowers on inflorescence. Suppressed bract primordia are present (arrowheads). **98.** Mature tepal tip bearing papillae. **99.** Enlarging carpel. The cleft extends down the adaxial side but not to the carpel base. The cleft is open on top of the young carpe. **100**. Mature carpel showing the short stipe and the punctiform stigma. **101.** Mature carpel base lacking glands. The cleft does not extend to the carpel base. **102.** Fusiform, papillate, capitate stigma. **103**. Partially dissected ovary showing the two orthotropous ovules in the locule. **Scale bars**: 93 = 500 μm; 94-95 = 25 μm; 96-98, 100, 101 = 100 μm; 99 = 250 μm.



Figs. 2.93-103

remains open distally (Fig. 2.99), but finally closes (Fig. 2.100). At maturity there is a short stipe (Figs. 2.100, 101). Papillae differentiate on the inner margins of the stigma (Fig. 2.102). Two orthotropous ovules with elongate funiculi develop within the locule (Fig. 2.103). At maturity, the outer integument does not cover the inner integument entirely. There are no visible signs of nectaries in these flowers (Fig. 2.101).

DISCUSSION

Floral Organogenesis - Conservation in organogenesis - In general, the initiation of floral organs is acropetal among taxa of Persoonioideae. The tepals are initiated in a conserved sub-opposite decussate pattern: the two frontal tepals initiate first and second followed by the sequential initiation of the sagittal tepals. After tepal initiation, the four stamens are initiated in the same sequence as the tepals, each stamen initiated opposite a tepal lobe. After stamen initiation, the floral meristem expands and enlarges for a period as it is converted into a terminal carpel.

The pattern of initiation of the tepals with the first two tepals in frontal positions has been termed eprophyllate aestivation (Weberling, 1989). That is, the first two organs in a flower are positioned analogously to the position of two prophylls on an axillary bud. Although the flowers of Proteaceae are four-merous and valvate, which often implies simultaneous initiation (as reported for: Oleaceae, Rhamnaceae; Sattler, 1973; some Amentiferous taxa MacDonald, 1971; some Mimosoid legumes, Ramírez-Domenech and Tucker, 1990), the tepals in flowers of Persoonioideae are initiated in sequential pairs. There is only a short interval (plastochron) between the initiation of sequential tepal primordia comprising each pair. There is a long plastochron between the tepals in different planes. The order in a flower is: frontal tepal - short plastochron -

opposite frontal tepal - long plastochron - sagittal tepal - short plastochron - opposite sagittal tepal.

Phyllotactically, the tepals represent two dimerous whorls of successive primordia. Similar patterns of perianth phyllotaxis in four-merous flowers have been reported in Lauraceae (Endress, 1972); Myrtaceae (Payer, 1857; Drinnan and Ladiges, 1988, 1989a-c, 1991a-b), male flowers of Quercus (Fagaceae) (MacDonald, 1971); Urticaceae (Sattler, 1973) four-merous Papaveraceae (Sattler, 1973; Karrer, 1991; Lehmann and Sattler, 1993), Brassicaceae (Sattler, 1973; Endress, 1992) some Potamogetonaceae (Charlton and Posluszny, 1991), Onagraceae (Sattler, 1973), and Buxaceae (pers. obs.; Drinnan, pers.comm.).

Are there taxonomically conserved patterns of tepal organogenesis among proteaceous taxa?- Consistent difference in tepal initiation involves which sagittal tepal initiates first. In flowers of taxa of Persooniinae, the adaxial tepal is initiated prior to the abaxial tepal. In Placospermum the abaxial tepal precedes initiation of the adaxial tepal. The sequential initiation of the sagittal tepals appears to be taxon specific and does not vary in different flowers of the same taxon as it does in the case of the frontal tepals.

Variation in the initiation of the frontal tepals - There is variation in the location of the first tepal to be initiated. The first tepal to become evident on the flanks of the floral meristem varies between the left or the right side of the floral meristem among flowers on different inflorescences, and sometimes on the same inflorescence. Asynchrony between primordia in decussate patterns is the general or usual case in shoots and flowers (Schoute, 1913; Rutishauser, 1981).

Tepal number variation - Tepal number varies among flowers of P. falcata. In most flowers, four tepals are initiated. In some flowers a fifth tepal is initiated in the adaxial half of the flower, between the third and first tepal. Two forms of meristic

variation are possible in flowers; a reduction of members in a whorl or an increase in number of organs per whorl. Meristic variation can occur within a species (infra-specific variation) or between higher taxonomic groups (supra-specific variation); infra-specific variation is generally less common than supra-specific meristic variation. ***Supra-specific reduction*** of organs is common in the sepal whorl of diverse genera of Scrophulariaceae (Kampny and Canne-Hilliker, 1987; Canne-Hilliker, 1987; Armstrong and Douglas, 1989; Douglas, 1989) both perianth whorls of species and genera of Leguminosae (Tucker, 1987, 1988, 1991), and stamens and carpels among both Piperaceae and Saururaceae (Tucker, 1984; Tucker, Douglas and Liang, 1993). ***Infra-specific reduction*** of the number of perianth members occurs in some mimosoid legumes (five to four; Ramìrez-Domenech and Tucker, 1990); some Gleditsia species (Tucker, 1991); some Compositae (Harris, 1991) and in Linanthus (Heuther, 1969).

Supra-specific increases in number of perianth members per whorl has been reported by Friis and Endress (1990) in different taxa of Crassulaceae and among some magnoliids, (Erbar and Leins, 1981). Endress (1990) suggested that the general variation of perianth numbers among some magnoliids (compared to the constrained number of perianth members in other taxa) is probably due to a lack of synorganiztion within the flowers. ***Infra-specific increases*** in number of perianth organs per whorl occurs in Ceratonia (Tucker, 1992), Gleditsia (Tucker, 1991), Linanthus (Heuther, 1969), and Ipomopsis (Ellstrand, 1983). Usually, in any given species, meristic variants will be present, however, the frequency of their occurrence is generally quite low, between 1-10% (Heuther, 1969; Ellstrand, 1983). The underlying mechanisms involved with intra-specific meristic variants have been hypothesized to be a combination of genetic and environmental factors (Heuther, 1969, Ellstrand, 1983; Heslop-Harrison and Wood, 1959). Heslop-Harrison and Wood (1959) induced meristic variation in Cannabis sativa by altering the temperatures (lower temperatures were correlated with a

greater number of meristic variants). The developmental events that underlie such meristic variants remain to be determined.

Among flowers of Persoonia falcata used in this study, 35% of the flowers are five-merous. In the five-merous flowers there is a change in phyllotaxis from a 1:4 pattern, as in most proteaceous taxa (Lucas series, not a Fibonacchi series number; Endress, 1987), to a 1:5 pattern including the stamens. An ontogenetic change in phyllotaxis from decussate to spiral has been suggested to result from an increase in the size of the apex prior to organogenesis (Cutter, 1965). Meicenheimer (1979, 1981, 1982) has identified two types of geometric changes in apices associated with transitional phyllotactic patterns during the the developmental transition to flowering: in some Ranunculus species, the sequential position of primordia on the apex is associated with the amount or volume of meristematic space (Meicenheimer, 1979); and in Epilobium hirsutum, different numbers of primordia cause apical shape changes (Meicenheimer, 1981, 1982). Stebbins (1974) asserts that changes in the number of organs are related to the the amount of meristematic cells in an apex relative to the number of meristematic cells necessary for the initiation and development of a primordium. In the five-merous flowers of P. falcata, there appears to be variation in the shape of the floral meristem, the amount of meristematic space and the positions of primordia compared with those of four-merous flowers (Figs. 2.58-59). A detailed examination of P. falcata will be conducted at a later date.

Antetepaly or super-positioning of the stamens and tepals - In the majority of angiosperm flowers, organs of the subsequent whorls generally alternate with those of the preceding whorls (organs are arranged in parastichies). Among flowers of Proteaceae, a single stamen is borne opposite to or superposed to each of the four tepals instead of alternating with the tepal whorl. Organs are arranged in orthostichies. The sequential initiation of the stamens is identical to the pattern of tepal initiation in that the

frontal stamens are initiated prior to the sagittal stamens. In all examined taxa, there are no vestiges of an alternating whorl of organs between the stamen and tepal whorl. Superposed stamens and perianth members are not uncommon among angiosperms. Lacroix and Sattler (1988) reported that superpositioning occurs among members of 13% of the angiosperm families. Lacroix and Sattler (1988) distinguished three types of super-positioned flowers. In the first group, a stamen primordium is initiated opposite each perianth member a common architecture among Potamogetonaceae (Charlton and Posluszny, 1991; Posluszny, 1993) Basellaceae (Lacroix and Sattler, 1988) and Proteaceae examined here. A second form of superpositioning between anthers and perianth members occurs when the stamen and perianth member form from a common primordium or a single growth center (Berberidaceae - Eichler, 1878; Endress, 1987; Primulales - Sattler, 1967, 1973; Sundberg, 1982). A third type of superpositioning is found when the stamens are initiated and develop in "halfway" positions or between an alternating and opposite position in relation to the positions of perianth members (Leins and Schwitalla, 1985). Recent floral ontogenetic studies have added a fourth type of superpositioning. In some taxa of Aristolochiaceae, the inner perianth whorl and the outer whorl of six stamens are absent (loss via reduction of organs present in related taxa, Leins and Erbar, 1985). The first stamens to be initiated are opposite each perianth member (Leins and Erbar, 1985; Leins, Erbar and Van Heel, 1988; Tucker and Douglas, in press). Floral ontogenetic investigations of other angiosperm groups will undoubtedly reveal additional variations involved with super-positioning between perianth members and stamens.

The phyllotactic implications and mechanisms involved with superpositioned floral organs remain a mystery. In most theories of phyllotactic positioning, it is postulated that the subsequent primordia are initiated in positions due to influence of previously initiated primordia, or due to preexisting conditions of the apex.

Space or mechanical theories imply that the successive primordia are borne in the next available space or the largest space (Hofmeister, 1868; van Iterson, 1907; Snow and Snow, 1931, 1952). Considering that proteaceous flowers maintain a distichous phyllotaxis up to the time of carpel initiation, the greatest amount of space of the apex is always in a perpendicular plane to the previously generated dimerous organ whorl.

In the "hélices foliaires" theory (Plantefol, 1948; elaborated by Loiseau, 1969 in Schwabe, 1984), it is postulated that there are multiple generative spirals in the apex or at the periphery of the apex in the Meristeme d'attente zone; in the case of proteacous flowers, there are four or two generative zones except in the five-merous flowers of P. falcata where there would be five zones.

In the inhibition or field theories, it is asserted that at initiation, an organ (or growth center) produces a field of inhibition that impedes and regulates the position of successive organ primordia (Schoute, 1913; Cutter, 1964; Schwabe, 1971, 1984; Gierer and Meinhardt, 1972; Mitchison, 1977; Young, 1978). Through successive plastochrons (the developmental interval between successive primordia), the field of inhibition decreases as the growth of the apex distances the successive primordia from the influence of the preceding primordia positioned lower down or away from the axis. Within this framework, the field of inhibition from successive primordia in a proteaceous flower has decreased or has been distanced from the sites of stamen initiation after four plastochrons (or two long ones in the case of two dimerous whorls), and thus the first organ has no affect by the time of stamen initiation. Schwabe (1971, 1984) proposed that there is basipetal polar transport of the inhibitor (inhibiting field) and that the expansion of the apex creates space between the apex and the preceding primordia. Lacroix and Sattler (1988) favoured the field-inhibition models to explain superpositioning, although they further suggest that additional experimental phyllotactic

studies are necessary prior to an accurate interpretation of the developmental mechanisms involved with super-positioned organs.

Ontogenetic variation in stamens among taxa - Considering that the stamen primordia are initially free at inception, adnation can be characterized as a generally derived character. A major difference observed in the development of the stamens and tepals among the taxa is the totally free filaments of Bellendena versus the adnate stamens of the other taxa. The morphogenetic events involved with the anthers after initiation differ. Among stamens of Garnieria, P. myrtilloides, and Bellendena, there is little or no elongation of a connective appendage. A connective appendage is however present in both Placospermum and Persoonia falcata, but the development of the appendage differs between the two taxa. In Placospermum, the connective appendage appears to be a product of early elongation after the differentiation of the microsporangia (Figs. 2.13, 15). In P. falcata, the connective appendage appears to form prior to and elongates during the differentiation of the microsporangia (Figs. 2.62, 64). The different developmental timing (heterochrony) of the connective appendages between P. falcata and Placospermum suggests convergence.

When is a carpel initiated? - There is ambiguity as to when, among unicarpellate angiosperms, the remaining floral meristem should be considered a carpel primordium (Tucker and Gifford, 1966 a, b; Sampson and Kaplan, 1970). Anatomical changes can occur before any morphological changes are expressed (Tucker and Gifford, 1966a, b; Sampson and Kaplan, 1970; Endress, 1972; Derstine and Tucker, 1991). The anatomical changes associated with the inception of the carpel primordium in some Persoonia species has been investigated. In both P. myrtilloides and P. falcata, the floral apex after stamen initiation enlarges and has a two layered tunica-corpus organization. Initiation occurs just before and during the morphological change in the shape of the floral apex from a bisymmetrical structure to a zygomorphic structure. A similar

transition was found in carpel initiation in flowers of Pseudowintera traversii and P. axillaris (Sampson and Kaplan, 1970).

Ontogenetic variation in carpels among taxa - Carpels differ developmentally among the taxa at virtually all developmental stages, as shown diagramatically (Fig. 2.104). In Placospermum (Fig. 2.104A), the formation of the cleft extends to the adaxial base of the carpel: in the other taxa, the cleft does not extend to the base (104B). Ensuing enlargement of the carpel differs as well. In Placospermum, the elongation of the carpel appears greater on the adaxial side than on the abaxial side, resulting in the cleft continuing from the adaxial side to the abaxial side of the flower (Fig. 2.104B-C). The bifid stigma develops on the abaxial side of the carpel along the suture margins (Fig. 2.104D). Like Placospermum, the stigma in Persoonia falcata is positioned abaxially (104I). In P. falcata the adaxial side elongates more than the abaxial side (104G-H). Two developmental differences between the carpels of Placospermum and Persoonia falcata is that in P. falcata the abaxial position of the stigma is enhanced by the curvature of the upper portion of the style (104H), and the stigmatic area is capitate as in other Persooniinae. In Garnieria and P. myrtilloides (Fig. 2.104K-M; as well as P. mollis and P. oblongata, not illustrated) circumferential enlargement at the top of the carpel results in a capitate stigma like P. falcata. The stigma in Bellendena is punctiform and there is no enlargement of the stigmatic surface.

The amount of development of a stipe differs among the taxa examined (Fig. 2.104D, I, L, M, O). A disk or cushion at the base of the enlarging carpel forms in all taxa of Persooniinae examined (Fig. 2.104F). In P. myrtilloides (Fig. 2.104M; as well as P. mollis and P. oblongata, not illustrated) the stipe becomes elongate and is narrow in relation to the remainder of the carpel. The stipe in Garnieria (Fig. 2.104L) is not as long or as narrow as P. myrtilloides. In P. falcata (Fig. 2.104I), the stipe is short.

Fig. 2.104. Diagram of different developmental processes responsible for gynoecium diversity in Persoonioideae. The **columns 1-6** represent different morphogenetic events in the different taxa arranged. **Column 1** is shows the common starting point, the enlarged floral meristem/carpel primordium before the formation of the cleft. **Column 2**. The development of the cleft on the adaxial side of the carpel primordium occurs in all taxa. However, the cleft extends to the base of the carpel in Placospermum (A) compared to the other taxa. **Column 3**. Enlargement and growth of the carpel primordium result in the cleft extending over the summit of the carpel in all taxa except possibly Bellendena montana. Basal to the carpel, a disk develops (solid black) in taxa of Persooniinae (F). **Column 4**. Continued enlargement of the carpel. The suture extends across the summit of the carpel and partially down the abaxial/dorsal side of the carpel in Placospermum (C) and Persoonia falcata (G). **Column 5**. Circumferential enlargement of upper portion of carpel (hoop with arrows) resulting in capitate stigma in Persooniinae (H and K). In P. falcata (H), the upper portion of the style bends abaxially (curved arrow). **Column 6**. The mature forms of the carpels (not drawn to scale) showing the abaxially positioned stigmas of Placospermum (D) and Persoonia falcata (I), the capitate stigmas, disks, stipes (different lengths), of Persooniinae (L, M) and the simple carpel of Bellendena montana (O). The locules of the different taxa are also depicted to show ovule number and position of the base of the carpel in relation to the locules. D=Placospermum coriaceum; I=Persoonia falcata; L=Garnieria spathulifolia; M=Persoonia myrtilloides, P. mollis, P. oblongata; O=Bellendena montana.

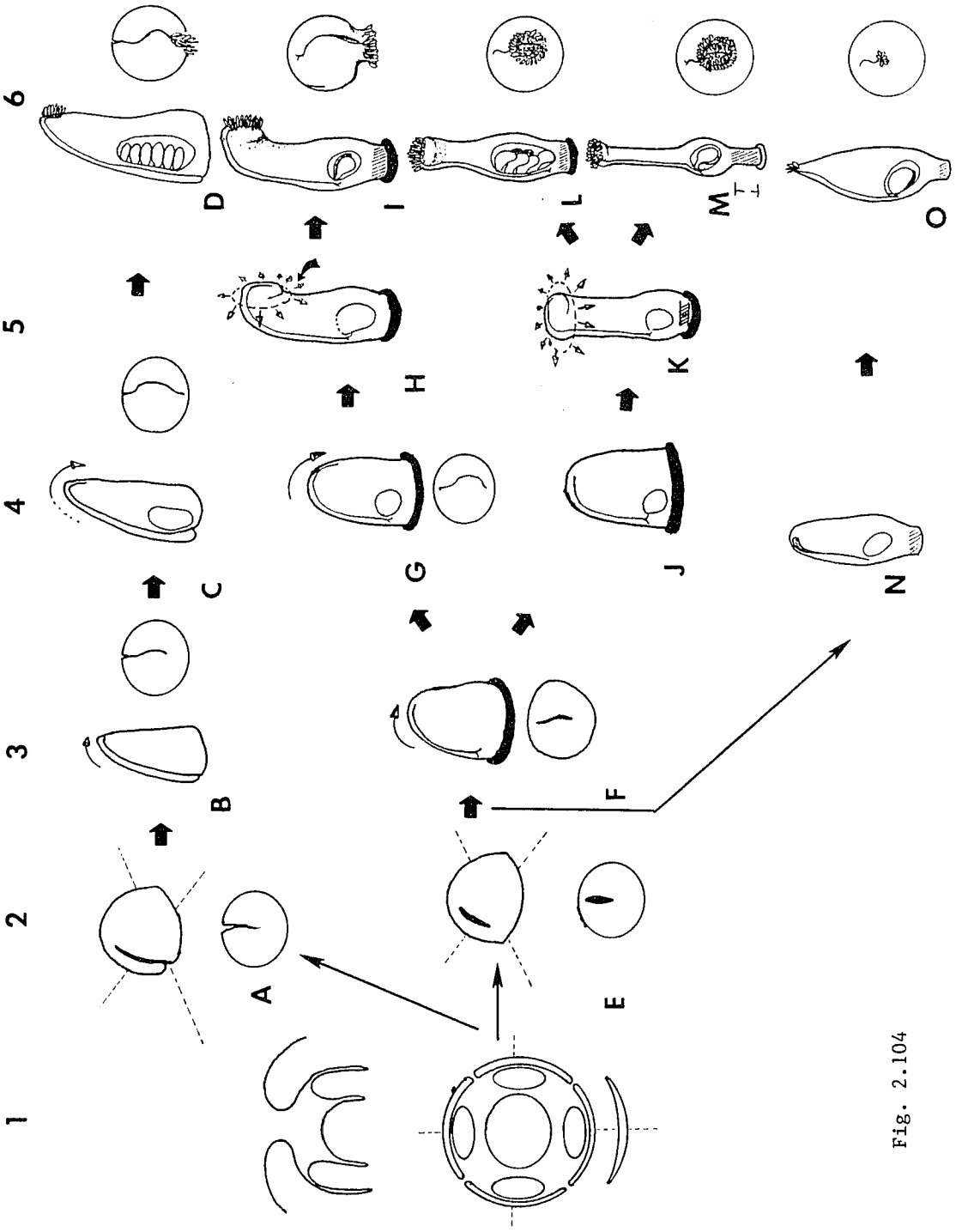


Fig. 2.104

Floral diversity in symmetry - Flowers among the taxa examined express zygomorphy in various ways at different developmental times and in differing floral organs. It should be noted that the presence of a single carpel in proteaceous taxa makes the flower topographically dorsiventral.

Zygomorphy is expressed in the tepals of some Persoonioideae. In flowers of Garnieria, P. myrtilloides, and Bellendena, the tepals are approximately equal in size and are equally reflexed at anthesis. In Placospermum, the adaxial tepal is slightly longer than the other three tepals, and in Persoonia falcata there is differential curvature among the tepals, the adaxial lobe being convex, the abaxial lobe being concave. Zygomorphy can be expressed in the androecium. In flowers of Garnieria, P. myrtilloides, P. falcata, and Bellendena, the four fertile anthers appear to be equally positioned in relation to the longitudinal floral axis. In flowers of Placospermum, the lateral and abaxial anthers are sterile.

Homology of the perianth - A single whorl of four tepals is borne in all investigated Persoonioideae (exception in some pentamerous flowers of P. falcata) and the other four subfamilies (Douglas, in prep.). Whether the single whorl of four free perianth lobes in Proteaceae is structurally homologous to the perianth parts of other angiosperms is a source of controversy. Interpretations and assumed homology of the proteaceous perianth has led to uncertainty about affinities with other angiosperms. Conflicting interpretations of the proteaceous perianth include homology to petals (Venkata Rao, 1971); sepal identity via loss of petals (Kausik, 1938, 1940, 1941; Eames, 1961; Haber, 1959, 1961, 1966; Venkata Rao, 1971); or tepals (primitively monochlamydeous or pre-Rosidae, Johnson and Briggs, 1975). Developmental studies show that there are no additional organs or vestigial organs initiated in the flowers of Proteaceae. The tepals provide protection for the

internal fertile organs, characteristic of sepals in other angiosperms. The tepals also are showy in many cases and the perigon can function as a synorganized tube, both characteristics of some petals in different angiosperms. A single trace (which usually splits into three) departs from the floral stele in all taxa (Venkata Rao, 1960, 1967, 1971).

Based on phylogenetic evidence, it has become apparent that Proteaceae is a relatively early offshoot of the eudicot lineage-"lowest hamamelids" (Chase, et al, 1993). The sister taxon to the family (Sabiaceae sensu stricto) has flowers with two perianth whorls that comprise a serial grade between bract-like to sepal-like to petal-like. Similar morphological variation in successive perianth lobes has been found in other basal angiosperms including Lauraceae (Endress, 1972), Schisandraceae (Tucker and Bourland, in press) and Nelumbonaceae (pers. obs.). It can therefore be asserted that Proteaceae is an early offshoot of a monochlamydeous line of angiosperms, and the four-merous flowers represent an early canalisation of constrained organ numbers. The assertion that the proteaceous flower is derived from a monochlamydeous apocarpous lineage is not new. Engler and Prantl (1894), Wettstein (1935) and Rendle (1938) suggested Proteaceae is primitively simple: as part of the Amentiferae theory of Engler and Prantl (1894), and as a primitively tepallous group from a reduced strobiloid type ancestor (Proberberidaceae) of Wettstein (1935). The evidence is compelling that the single perianth whorl in Proteaceae is primitively or ancestrally simple.

What is the spine on the tepals? - A spine or elongation is present on the tepals of Persoonieae examined. This structure has been termed a **Vorläuferspitze** or the precursor tip (Kaplan, 1973) and has been observed on the perianth lobes of several angiosperm groups (Baum, 1951, Baum-Leinfellner, 1953). Kaplan (1970, 1973) reported similar structures in the leaves of most monocotyledons and phyllodes of various dicotyledons (e.g. Acacia). Weberling (1989) refers to such structures as

corniculations. The Vorläuferspitze in flowers tends to occur on the outer most perianth members or sepals in most cases. No known function has been associated with Vorläuferspitze although its size and consistent presence in taxa of Persoonieae (particularly on the adaxial tepal of Placospermum) provides several questions from both a developmental and evolutionary point of view. A Vorläuferspitze is also found in some members of other proteaceous subfamilies; Petrophile (Proteoideae) and Darlingia (Grevilleoideae). In leaves, the Vorläuferspitze is the residual apex of the leaf primordium (Kaplan, 1973, 1975). Although, not anatomically investigated, a similar process could be occurring in the development of the spine in proteaceous flowers as well as those of other angiosperms (Baum, 1951; Baum-Leinfellner, 1953).

Homology of the nectaries - Adding to the ambiguity of the interpretation of the perianth are nectariferous glands that are present in alternitepalous positions in the mature flowers. Differing opinions of the homology of the glands include petal homologues (Kausik, 1938, 1940, 1941; Eames, 1961; Haber, 1959, 1961, 1966), and functional enations that are not homologous to any of the primary floral organs (Venkata Rao, 1967, 1971; Johnson and Briggs, 1975). Developmental studies among Persoonieae clearly illustrate that the nectaries are initiated internal to the stamen whorl after the formation of all of the organs and usually well after the other floral organs have begun to mature. They are in the wrong place for petals, inside the stamen whorl. In addition, if the nectaries were homologous to a reduced perianth whorl, one would expect to observe spaces in the alternitepalous sites or even in alternistamenous sites. In fact, the entire floral meristem changes into a carpel primordium. Spaces for the development of the nectaries appears after zonal growth between the tepals and stamens has lifted the anthers and broadened the floral receptacle. Most floral nectaries in fact are initiated well after all floral organs are present (Tucker, 1987).

Are the nectaries petals?- The idea that the nectaries among Proteaceae represent reduced perianth homologues should finally be abandoned. The nectaries are no more than functional enations. Anatomical studies have illustrated that vascularization patterns are diverse among the nectaries in different taxa of Proteaceae, including the nectary trace originating from the lateral tepal traces, the median tepal traces, the carpel trace and in some taxa the stamen traces (Kausik, 1938, 1941; Haber, 1959, 1961, 1966; Venkata Rao, 1960, 1961, 1967, summarized in 1971). In addition, the nectaries in some taxa lack vascular traces entirely and still others have what Venkata Rao (1967) termed phloem-like cells. The multiple patterns of vascular supply of the nectaries led Venkata Rao (1967, 1971) to the conclusion that nectaries have been derived multiple times among proteaceous taxa. Venkata Rao (1971) felt that the nectaries are derived within the family and that Bellendena, that lacks nectaries, represents the simplest and probably most archaic member of the family. Johnson and Briggs (1975) concluded that since 65 of 75 genera in the family have some form of nectary, a nectary was probably present in a common ancestor to the family. Phylogenetic conclusions concerning the origin of the proteaceous nectary will be the focus of another study. It should however be noted that th phylogenetic analysis of angiosperms by Chase et al. (1993) suggested Sabia (Sabiaceae sensu stricto) had a common ancestor with Proteaceae. Sabia flowers similarly have nectaries borne alternating with the other floral organs that are arranged orthostichously as in proteaceous flowers (Douglas, pers. obs.; Heywood, 1993).

Inflorescence and plant architecture variation - The inflorescences of tribes Persoonieae and Bellendeneae are morphologically and fundamentally different. In Bellendena (Bellendeneae) the position of the inflorescence is auxotelic, and terminates the growth of the vegetative axis. Termination of the main axis with an inflorescence was occasionally observed in Placospermum

(Placosperminae, Persoonieae). In the other taxa of Persoonieae examined (Persooniinae), the main axis is anauxotelic.

The type of inflorescence is diverse in Persoonioideae. The inflorescences include unbranching racemes with internode elongation between flowers, (some Persoonia spp. and Garnieria), simple condensed racemes with little or no elongation between flowers (Bellendena), compound or branching racemes (Placospermum) and in some Persoonia species, single flowers are borne in leaf axils along indeterminate shoots (flowering branches). Such fundamental differences in architecture suggest distant relationships among subtribes and tribes of Persoonioideae or rapid rates of morphological divergence in relation to inflorescence diversity.

LITERATURE CITED IN CHAPTER 2

Armstrong, J. E. and A. W. Douglas. 1989. The ontogenetic basis for corolla aestivation in Scrophulariaceae. Bulletin of the Torrey Botanical Club 116: 378-389.

Baum, H. 1951. Vorläuferspitzen im Blütenbereich. Österreiche Botanische Zeitschrift 98: 280-291.

Baum-Leinfellner, H. 1953. Über unifaziale Griffel und Narben. Planta 42: 452-460.

Briggs, B. G. and L. A. S. Johnson. 1979. Evolution in the Myrtaceae - Evidence from inflorescence structure. Proceedings of the Linnean Society of New South Wales 102: 157-272.

Canne-Hilliker, J. M. 1987. Patterns of floral development in Agalinis and allies (Scrophulariaceae): II. Floral development of Agalinis densiflora. American Journal of Botany 74: 1419-1430.

Charlton, W. A. and U. Posluszny. 1991. Meristic variation in Potamogeton flowers. Botanical Journal of the Linnean Society 106: 265-293.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y-L Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedren, B. S. Gaut, R. K. Jansen, K-J Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q-Y Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. William, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan, and V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene rbcL. Annals of the Missouri Botanical Garden 80: 526-579.

Cutter, E. 1964. Phyllotaxis and apical growth. The New Phytologist 63: 39-46.

Cutter, E. 1965. Recent experimental studies of the shoot apex and shoot morphogenesis. The Botanical Review 31: 7-113.

Derstine, K. S. and S. C. Tucker. 1991. Organ initiation and development of inflorescences and flowers of Acacia baileyana. American Journal of Botany 78: 816-832.

Douglas, A. W. 1989. The ontogenetic basis of diverse floral morphologies in Pedicularis L. (Scrophulariaceae). M.S. Illinois State University, Normal, Illinois.

Drinnan, A. N. and P. Y. Ladiges. 1988. Perianth development in Angophora and the Bloodwood Eucalypts (Myrtaceae). Plant Systematics and Evolution 160: 219-239.

Drinnan, A. N. and P. Y. Ladiges. 1989a. Operculum development in the Eudesmia B eucalypts and Eucalyptus caesia (Myrtaceae). Plant Systematics and Evolution 165: 227-237.

Drinnan, A. N. and P. Y. Ladiges. 1989b. Corolla and androecium development in some Eudesmia eucalypts (Myrtaceae). Plant Systematics and Evolution 165: 239-254.

Drinnan, A. N. and P. Y. Ladiges. 1989c. Operculum development in Eucalyptus clöezmia and Eucalyptus informal subg. Monocalyptus (Myrtaceae). Plant Systematics and Evolution 166: 183-196.

Drinnan, A. N. and P. Y. Ladiges. 1991a. Floral development and systematic position of Eucalypus curtisii (Myrtaceae). Australian Systematic Botany 4: 539-551.

Drinnan, A. N. and P. Y. Ladiges. 1991b. Floral development in the 'Symphyomyrtus Group' of Eucalypts (Eucalyptus: Myrtaceae). Australian Systematic Botany 4: 553-562.

Eames, A. J. 1961. Morphology of the Angiosperms. McGraw-Hill Book Company, New York.

Eichler, A. W. 1878. Blüthendiagramme II. Otto Koeltz, Eppenhain.

Ellstrand, N. C. 1983. Floral formula inconstancy within and among plants and populations of Ipomopsis aggregata (Polemoniaceae). Botanical Gazette 144: 119-123.

Endress, P. K. 1972. Zur vergleichenden Entwicklungsmorphologie, Embryologie und Systematik bei Laurales. Botanische Jahrbücher der Systematik 92: 331-428.

Endress, P. K. 1977. Evolutionary trends in the Hamamelidales-Fagales group. Plant Systematics and Evolution, Supp. 1: 321-347.

Endress, P. K. 1987. Floral phyllotaxis and floral evolution. Botanische Jahrbücher der Systematik 108: 417-438.

Endress, P. K. 1990. Patterns of floral construction in ontogeny and phylogeny. Biological Journal of the Linnean Society 39: 153-175.

Endress, P. K. 1992. Evolution and floral diversity: The phylogenetic surroundings of Arabadopsis and Antirrhinum. International Journal of Plant Sciences 153 (part 2): 106-122.

Engler, A. and K. Prantl. 1894. Die Natürlichen Pflanzenfamilien. Leipzig. Excerpt on Proteaceae by A. Engler (1893), Vol IV.

Erbar, C., and P. Leins. 1981. Zur Spirale in Magnolien-Blüten. Beiträge zur Biologie der Pflanzen 56: 225-241.

Friis, E. M. and P. K. Endress. 1990. Origin and evolution of angiosperm flowers. Advances in Botanical Research 17: 99-162.

Gierer, A. and H. Meinhardt. 1972. A theory of biological pattern formation. Kybernetik 12: 30-39.

Grimes, J. 1992. Metamerism, heterochrony, and inflorescence morphology of the Pithecellobium-complex (Leguminosae: Mimosoideae: Ingeae). Brittonia 44: 140-159.

Haber, J. M. 1959. Comparative anatomy and morphology of flowers and inflorescences of the Proteaceae I. Some Australian taxa. Phytomorphology 9: 325-358.

Haber, J. M. 1961. The comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae II. Some American taxa. Phytomorphology 11: 1-16.

Haber, J. M. 1966 Comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae III. Some African taxa. Phytomorphology 16: 490-527.

Harris, E. M. 1991. Comparative inflorescence and floral development in the Asteraceae. Ph.D. diss. Louisiana State University, Louisiana.

Heslop-Harrison, Y. and I. Wood. 1959. Temperature induced meristic and other variations in Cannabis sativa. Journal of the Linnean Society, London 56: 290-293.

Heuther, C. A., Jr. 1969. Constancy of the pentamerous corolla phenotype in natural populations of Linanthus. Evolution 23: 572-588.

Heywood, V. H. 1993. Flowering Plants of the World. Oxford University Press, Oxford.

Hofmeister, W. 1868. Allgemeine Morphologie der Gewächse. Engelmann, Leipzig.

Iterson, G. van, Jr. 1907. Mathematische und mikroskopische-anatomische Studien über Blattstellungen. Jena: Fischer Verlag.

Johnson, L. A. S. and B. G. Briggs. 1963. Evolution in the Proteaceae. Australian Journal of Botany 11: 21-61.

Johnson, L. A. S. and B. G. Briggs. 1975. On the Proteaceae--the evolution and classification of a southern family. Botanical Journal of the Linnaean Society 83-182.

Kampny, C. M. and J. M. Canne-Hilliker. 1987. Patterns of floral development in Agalinis and allies (Scrophulariaceae). I. Floral development of Agalinis fasciculata and A. tenuifolia. Canadian Journal of Botany 65: 2255-2262.

Kaplan, D. R. 1970. Comparative foliar histogenesis in Acorus calamus and its bearing on the phyllode theory of monocotyledonous leaves. American Journal of Botany 57: 331-361.

Kaplan, D. R. 1973. The monocotyledons: their evolution and comparative biology. VII. The problem of leaf morphology and evolution in the monocotyledons. The Quarterly Review of Biology 48: 437-457.

Kaplan, D. R. 1975. Comparative developmental evaluation of the morphology of unifacial leaves in the monocotyledons. Botanische Jahrbücher der Systematik 95:1-105.

Karrer, A. B. 1991. Blütentwicklung und Systematische Stellung der Papaveraceae und Capparaceae. Ph.D. Dissertation, University of Zürich, Zürich.

Kausik, S. B. 1938. Studies in the Proteaceae. II. Floral anatomy and morphology of Macadamia ternifolia. Proceedings of the Indian Academy of Sciences, 8B: 45-62.

Kausik, S. B. 1940. Vascular anatomy of the flower of Macadamia ternifolia F. Muell. Current Science 9: 22-25.

Kausik, S. B. 1941. Studies in the Proteaceae. VI. Vascular anatomy of the flower of Grevillea robusta. Proceedings of the National Institute of Sciences of India 7: 257-266.

Kunze, von, Henning. 1978. Typologie und Morphogenese des Angiospermen-Staubblattes. Beiträge zur Biologie der Pflanzen 239 -304.

Lacroix, C. and R. Sattler. 1988. Phyllotaxis theories and tepal-stamen superposition in Basella rubra. American Journal of Botany 75: 906-917.

Lehmann, N. L. and R. Sattler. 1993. Homeosis in floral development of Sanguinaria canadensis and S. canadensis 'Multiplex' (Papaveraceae). American Journal of Botany 80: 1323-1335.

Leins, P. and S. Schwitalla. 1985. Studien an Cactaceen-Blüten. I. Einige Bemerkungen zur Blütenentwicklung von Pereskia. Beitrage der Biologische Pflanzen 60: 313-323.

Leins, P., and C. Erbar. 1985. Ein Beitrag zur Blütenentwicklung der Aristolochiaceen, einer Vermittlergruppe zu den Monokotylen. Botanische Jahrbücher für Systematik 107: 343-368.

Leins, P., C. Erbar, and W. A. Van Heel. 1988. Notes on the floral development of Thottea (Aristolochiaceae). Blumea 33: 357-370.

Loiseau, J. -E. 1969. La Phyllotaxie. Paris: Masson and Cie.

MacDonald, A. D. 1971. Floral development in the 'Amentiferae.' Ph. D. diss. McGill University, Montreal.

Meicenheimer, R. D. 1979. Relationships between shoot growth and changing phyllotaxy of Ranunculus. American Journal of Botany 66: 557-569.

Meicenheimer, R. D. 1981. Changes in Epilobium phyllotaxy induced by N-1-Naphthylphthalamic acid and alpha-4-chlorophenoxyisobutyric acid. American Journal of Botany 68: 1139-1154.

Meicenheimer, R. D. 1982. Change in Epilobium phyllotaxy during reproductive transition. American Journal of Botany 69: 1108-1118.

Mitchison, G. J. 1977. Phyllotaxis and the Fibonacci Series. Science, 196: 270-275.

Payer, J. B. 1857. Traité d'Organogénie comparée de la fleur. Paris: Librairie de Victor Masson.

Plantefol, L. 1948. La Théorie des Hélices Foliaires Multiples. Paris: Masson and Cie.

Posluszny, U. 1993. Evolution of the helobial flower. Aquatic Botany 44: 303-324.

Ramírez-Domenech, J. I. and S. C. Tucker. 1990. Comparative ontogeny of the perianth in mimosoid legumes. American Journal of Botany 77: 624-635.

Rendle, A. B. 1938. Classification of Flowering Plants. Vol. II. Dicotyledons. Cambridge.

Rutishauser, R. 1981. Blattstellung und Sprossentwicklung. Dissertationes Botanicae 62. Pp. 127, 18 plates. Vaduz: Cramer.

Sakai, W. S. 1973. Simple method for differential staining of paraffin embedded plant material using toluidine blue O. Stain Technology 48: 247-249.

Sampson, F. B. and D. R. Kaplan. 1970. Origin and development of the terminal carpel in Pseudowintera traversii. American Journal of Botany 57: 1185-1196.

Sattler, R. 1967. Petal inception and the problem of pattern detection. Journal of Theoretical Biology 17: 31-39.

Sattler, R. 1973. Organogensis of flowers. A photographic text-atlas. University of Toronto Press, Toronto.

Schoute, J. C. 1913. Beiträge zur Blattstellungslehre. I. Die Theorie. Recueil des Travaux Botanique Néerlandais 10: 153-325.

Schwabe, W. W. 1971. Chemical modification of phyllotaxis and its implications. Symposia of the Society for Experimental Biology 25: 301-322.

Schwabe, W. W. 1984. Phyllotaxis. Pages 403-440 *in* P.W. Barlow and D.J. Carr, eds. Positional controls in plant development. Cambridge University Press, Cambridge.

Snow, M. and R. Snow. 1931. Experiments on phyllotaxis. I. The effect of isolating a primordium. Philosophical Transactions of the Royal Society of London, B, 221: 1-43.

Snow, M. and R. Snow. 1952. Minimum area and leaf determination. Proceedings of the Royal Society of London, B, 139: 545-566.

Snow, M. and R. Snow. 1959. Regulation of sizes of leaf primordia by older leaves. Philisophical Transactions of the Royal Society of London, B 151: 39-47.

Stebbins, G. L. 1974. Flowering plants. Evolution above the species level. Harvard University Press, Cambridge.

Sundberg, M. D. 1982. Petal-stamen initiation in the genus Cyclamen (Primulaceae). American Journal of Botany 69: 1707-1709.

Tucker, S. C. 1984. Origin of symmetry in flowers. Pages 351-395*in*: R. A. White and R.C. Dickison, eds., Contemporary problems in plant anatomy. Academic Press, New York.

Tucker, S. C. 1987. Floral initiation and development in legumes. Pages 183-239*in:* C.H. Stirton, ed. Advances in legume systematics, Vol. III. Royal Botanic Gardens, Kew.

Tucker, S. C. 1988. Loss versus suppression of floral organs. Pages 69-82*In:* P. Leins, S.C. Tucker and P.K. Endress, eds. Aspects of floral development. J. Cramer, Berlin.

Tucker, S. C. 1991. Helical floral organogenesis in Gleditsia, a primitive caesalpinioid legume. American Journal of Botany 78: 1130-1149.

Tucker, S. C. 1992. The developmental basis for sexual expression in Ceratonia siliqua (Leguminosae: Caesalpinioideae: Cassieae). American Journal of Botany 79: 318-327.

Tucker, S. C. and J. Allen Bourland. In press. Ontogeny of staminate and carpellate flowers of Schisandra glabra (Schisandraceae). Plant Systematics and Evolution, Supp. 8.

Tucker, S. C. and A. W. Douglas. In press. Floral structure, development, and relationships of paleoherbs: Saruma, Cabomba, Lactoris, and selected Piperales. *In* L. Hickey and D. W. Taylor, eds. Flowering plant origin, evolution, and phylogeny.

Tucker, S. C. and E. M. Gifford, Jr. 1966a. Organogenesis in the carpellate flower of Drimys lanceolata. American Journal of Botany 53: 433-442.

Tucker, S. C. and E. M. Gifford, Jr. 1966b. Carpel development in Drimys lanceolata. American Journal of Botany 53: 671-678.

Tucker, S. C., A. W. Douglas and H. -X. Liang. 1993. Utility of ontogenetic and conventional characters in determining phylogenetic relationships of Saururaceae and Piperaceae (Piperales). Systematic Botany 18: 614-641.

Venkata Rao, C. 1957. Cytotaxonomy of the Proteaceae. Proceedings of the Linnaean Society, N.S.W. 82: 257-271.

Venkata Rao, C. 1960. Studies in the Proteaceae I. Tribe Persoonieae. Proceedings of the National Institute of Sciences of India 26B: 300-337.

Venkata Rao, C. 1961. Studies in the Proteaceae II. Tribes Placospermeae and Conospermeae. Proceedings of the National Institute of Sciences of India 27B: 126-151.

Venkata Rao, C. 1967. Morphology of the nectary in Proteaceae. New Phytologist 66: 99-108.

Venkata Rao, C. 1971. Proteaceae. Botanical Monograph #6. Council of Scientific and Industrial Research. New Delhi.

Weberling, F. 1989. Morphology of Flowers and Inflorescences. Cambridge University Press, Great Britain.

Weston, P. W. 1983. The systematics and biogeography of the Persooniinae (Proteaceae). Ph.D. diss. University of Sydney, N.S.W., Australia.

Weston, P. W. and L. A. S. Johnson. 1991. Taxonomic changes in Persoonia (Proeaceae) in New South Wales. Telopea 4: 269-306.

Wettstein, R. 1935. Handbuch der Systematischen Botanik, 4th ed. Leipzig.

Wrigley, J. W. and M. Fagg. 1988. Banksias, Waratahs and Grevilleas. Collins Publishers, Sydney, N.S.W.

Young, D. A. 1978. On the diffusion theory of phyllotaxis. Journal of Theoretical Biology 71: 421-432.

CHAPTER 3

COMPARATIVE FLORAL ONTOGENY OF PROTEOIDEAE (PROTEACEAE)

INTRODUCTION

Proteaceae is a moderate-sized family of approximately 75 genera and 1200 species. Members are found primarily in the southern hemisphere, with the greatest abundance of species and genera in Australia and the Malesian region (Johnson and Briggs, 1975). The flowers of Proteaceae maintain a relatively consistent and unique structure composed of a single whorl of four free tepals, four basifixed tetrasporangiate stamens, one borne opposite each tepal (antetepalous), and a single carpel. Johnson and Briggs (1975) divided the family into five subfamilies; Persoonioideae, Proteoideae, Sphalmioideae, Carnarvonioideae, and Grevilleoideae. Persoonioideae has been hypothesized to represent the basal clade of Proteaceae, based primarily on the diploid chromosome numbers (Venkata Rao, 1971; Johnson and Briggs, 1975). Proteoideae is composed of 26 genera and is the second largest subfamily after Grevilleoideae. Characteristics distinguishing the subfamily include flowers not in grevilleoid-pairs (two flowers per common bract; see Chapter 4), indehiscent fruit (sometimes drupes but usually dry achenes), one or two ovules, usually only one seed, and small chromosomes at the tetraploid level (Johnson and Briggs, 1975).

Proteoideae is divided into three tribes. **Conospermeae** includes 11 genera (Johnson and Briggs, 1975) and is divided into five subtribes. The first, Cenarrheniinae, is made up of five genera: two in New Caledonia (Beauprea, Beaupreopsis) and the other three in Australia, Cenarrhenes, Symphionema, and Agastachys. The second subtribe, Dilobeiinae, is unigeneric and found in Madagascar. Petrophilinae is composed of two speciose genera in Australia, Isopogon and Petrophile. Stirlingiinae is unigeneric and found in south-western Australia (Western Australia), and Conosperminae is composed of two Australian genera: Conospermum (eastern and western Australia) and Synaphea (W. Australia). The Australian tribe **Franklandieae** is

composed of two unigeneric subtribes, Adenanthiinae and Franklandiinae. The African tribe **Proteeae** is composed of 13 genera divided into two subtribes.

As part of an ongoing comparative project, the floral ontogenies of selected proteaceous taxa are being examined to determine the developmental basis of floral diversity within the family, as well as to better understand the nature of the highly canalised flowers. Evidence from comparative ontogenetic studies will ultimately be used to reexamine the classification of the family by Johnson and Briggs (1975). In this study, the floral ontogenies of representative members of Proteoideae are investigated and compared. Representative taxa studied include Symphionema montana, Isopogon formosus, Synaphea polymorpha, Conospermum caeruleum, Stirlingia latifolia, Adenanthos obovatus, Adenanthos oreophilous, and Serruria pedunculata.

METHODS AND MATERIALS

Taxa investigated and provenance are listed in Table 3.1. In all cases, fresh floral material was fixed in FAA (90 ml 50% ethanol; 5 ml glacial acetic acid; 5 ml 37% formalin). After fixation, material was stored in 50% ethanol. Floral material was microdissected in 95% ethanol with a Wild M2A dissecting scope using fiber optic illumination. Prepared materials were dehydrated with absolute alcohol and acetone and critical point dried using a Denton apparatus with liquid carbon dioxide. Dried materials were mounted on aluminum stubs with colloidal graphite and coated with approximately 100-500 angstroms of gold-palladium in either a Hummer II or a Technics sputter coater . Prepared material was examined with a Cambridge S-260 scanning electron microscope. Images were recorded on Kodak Tri-X Pan 4 x 5 film sheet film.

Terminology -- There are different terms associated with the different sides and planes of flowers. Terminology that will be used to compare proteaceous flowers includes the

Table 3.1. Species and provenance of species examined. **=examined but not illustrated in text.

Taxa	Native to:	Source(s)
Symphionema montana R. Br.	S. E. Australia	Royal Botanic Gardens (R.B.G.) Sydney at the Mt. Annan Gardens
**Cennarhenes nitida Labill.	Tasmania	Tasmania, (F. Podyer, coll.; CSIRO Queensland)
**Beaupreopsis paniculata (Brongn. and Gris.) Virot	New Caledonia	New Caledonia, (P. Weston, coll.)
**Agastachys odorata R. Br.	Tasmania	Tasmania (J. Chappill, coll.)
Conospermum caeruleum R. Br.	W. Australia	University of Calfifornia, Santa Cruz (U.C.S.C.) Arboretum; Western Australia.
Synaphea polymorpha R. Br.	W. Australia	R.B.G. Sydney- Mt. Annan Gardens
Adenanthos obovatus Labill.	S. W. Australia	U.C.S.C. Arboretum; Stirling Range, W. Australia.
Adenanthos oreophilus Nelson	S. W. Australia	R.B.G. Sydney
Isopogon formosus R. Br.	W. Australia	U.C.S.C. arboretum
**Isopogon cuneatus R. Br.	W. Australia	U.C.S.C. arboretum
**Petrophile serruriae R. Br.	S. W. Australia	U.C.S.C. arboretum
Serruria pedunculata R.Br.	South Africa	U.C.S.C. arboretum

median **sagittal** plane, that divides a flower and the subtending leaf (or bract) into mirror-image halves. The median **frontal** plane is perpendicular to the median sagittal plane. The **adaxial** side of the flower is the upper half, closest to the inflorescence axis, and the **abaxial** side is the lower half of the flower closest to the subtending leaf (or bract). There are different terms associated with the symmetry of flowers. **Actinomorphic** is the same as radial symmetry, in which the structure or flower has multiple planes of symmetry; **bisymmetrical** refers to structures that have two planes of symmetry, each dividing the structure or flower into two mirror-image halves (Weberling, 1989; Friis and Endress, 1990), and **zygomorphy** (dorsiventral) refers to structures or flowers that have a single line of symmetry; the abaxial and adaxial halves are unlike (Weberling, 1989; Friis and Endress, 1990).

The possible structural homology between the single whorl of four free perianth lobes in Proteaceae and the perianth of other angiosperms is a source of controversy. Conflicting interpretations of the proteaceous perianth include petals (Schacht, 1853; Venkata Rao, 1971 - in part); sepals after loss of petals (Kausik, 1936, 1941; Eames, 1961; Haber, 1961, 1966; Venkata Rao, 1971 - in part); and tepals (primitively monochlamydeous or pre-Rosidae, Johnson and Briggs, 1975). Ontogenetic comparisons of other proteaceous taxa (Chapter 2) shows that there is only a single whorl of perianth parts. Hence the perianth lobes are referred to as **tepals** because the perianth is unspecialized and has both petalline and sepalline qualities. **Perigon** is a collective term for the proteaceous perianth lobes (Johnson and Briggs, 1975; Weberling, 1989). The aestivation pattern of the tepals in Proteaceae is **valvate**, with margins in contact edge to edge. Aestivation of the tips of tepals varies among taxa. In most Proteaceae, the lateral tepal tips contact one another above the other floral organs. The sagittal tepals subsequently fill in the spaces between the lateral tepals and do not

contact one another. This will be referred to as **incomplete-valvate aestivation**. Variations from this pattern are discussed when they occur.

Each of the four stamens in a proteaceous flower is antetepalous, borne opposite a tepal lobe or superposed. Intercalary growth occurs between and beneath each tepal and stamen in most taxa, resulting in an **epitepalous** condition (the stamen is adnate to the tepal). There are several terms associated with the morphogenesis of the anthers. **Median furrow** refers to the furrow or crease that bisects the longitudinal axis of each anther, resulting in two equal halves, **thecae**. The **transverse furrow** refers to the furrow or crease that divides each theca into two separate **microsporangia** (Kunze, 1978).

Flowers of Proteoideae have a single terminal carpel. The entire floral apex, after stamen initiation, is utilized in the formation of the carpel. For the purpose of the following ontogenetic description, when the product of the floral meristem, after stamen initiation, becomes zygomorphic, it is termed a carpel primordium.

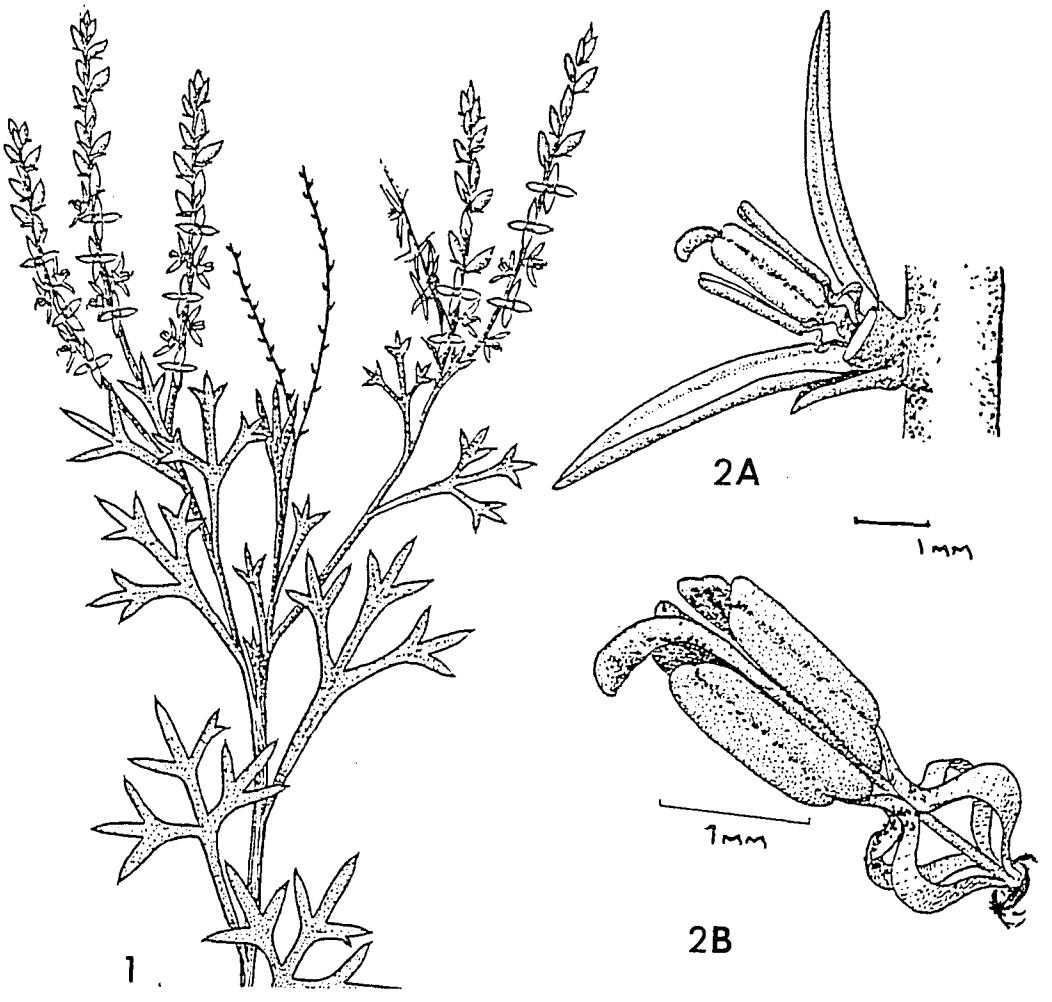
Inflorescences among members of Persoonioideae are variable in form. Terms for inflorescence morphology used here include **blastotelic,** an indeterminate inflorescence, and **anthotelic**, that is a determinate inflorescence and ends in a flower (Briggs and Johnson, 1979). Briggs and Johnson (1979) provided two additional terms for blastotelic inflorescences; **anauxotelic** inflorescences are blastotelic inflorescences in which growth does not continue beyond the flowering region; **auxotelic** inflorescences are blastotelic inflorescences where growth continues beyond the flowering region. It should be noted that auxotely and anauxotely are applicable to a shoot axis as well as parts of inflorescences (Briggs and Johnson, 1979; Grimes, 1992). A relative growth unit is the axis produced by a single meristem (Grimes, 1992). The arrangement of bract primordia in an inflorescence is termed **anthotaxis** and is

analagous to phyllotaxis, a term used for the arrangement of leaves on a shoot. The positions of bract primordia in relation to one another on the inflorescence lie along **anthotactic spirals**.

OBSERVATIONS

Conospermeae- Cenarrheniinae - Symphionema montana - Figs.1-29.- Organography - Plants of Symphionema montana are woody, sprawling shrubs found in southeastern Australia. The inflorescences are heterothetic indeterminate (blastotelic) compound racemes that branch up to four times in some specimens (Fig. 3.1; Weberling, 1989). Inflorescence branches and flowers are arranged in sub-opposite pairs with longer internodes between the pairs. The relative growth unit of the plant is anauxotelic and can be divided into two subsequences, a vegetative and a reproductive region (Fig. 3.1). The proximal portion of the axis is vegetative. Distally, there is a serial transition of successive leaves, from a lobed condition (typical for the vegetative body) to elongate, acutely tapered bracts below the inflorescence. Secondary inflorescence axes are produced in leaf or bract axils on proximal parts of the main inflorescence axis, that can produce tertiary inflorescence axes as well. Each inflorescence axis includes 15-25 subopposite positioned bracts with a single flower in each bract axil. The number of flowers on each inflorescence branch is approximately equal.

A sessile yellowish flower is borne within the axil of a bract (Fig. 3.2, 25). The four glabrous and petaloid tepals reflex equally from the floral base although the bract stands erect and restricts the path of reflexion of the the abaxial tepal at anthesis. In each of the four antetepalous stamens, the filament is adnate to the base of the tepal. The anthers are basifixed and introrse. There is no distal connective appendage above the parallel thecae. In bud, the four anthers are tightly appressed and fused to a small

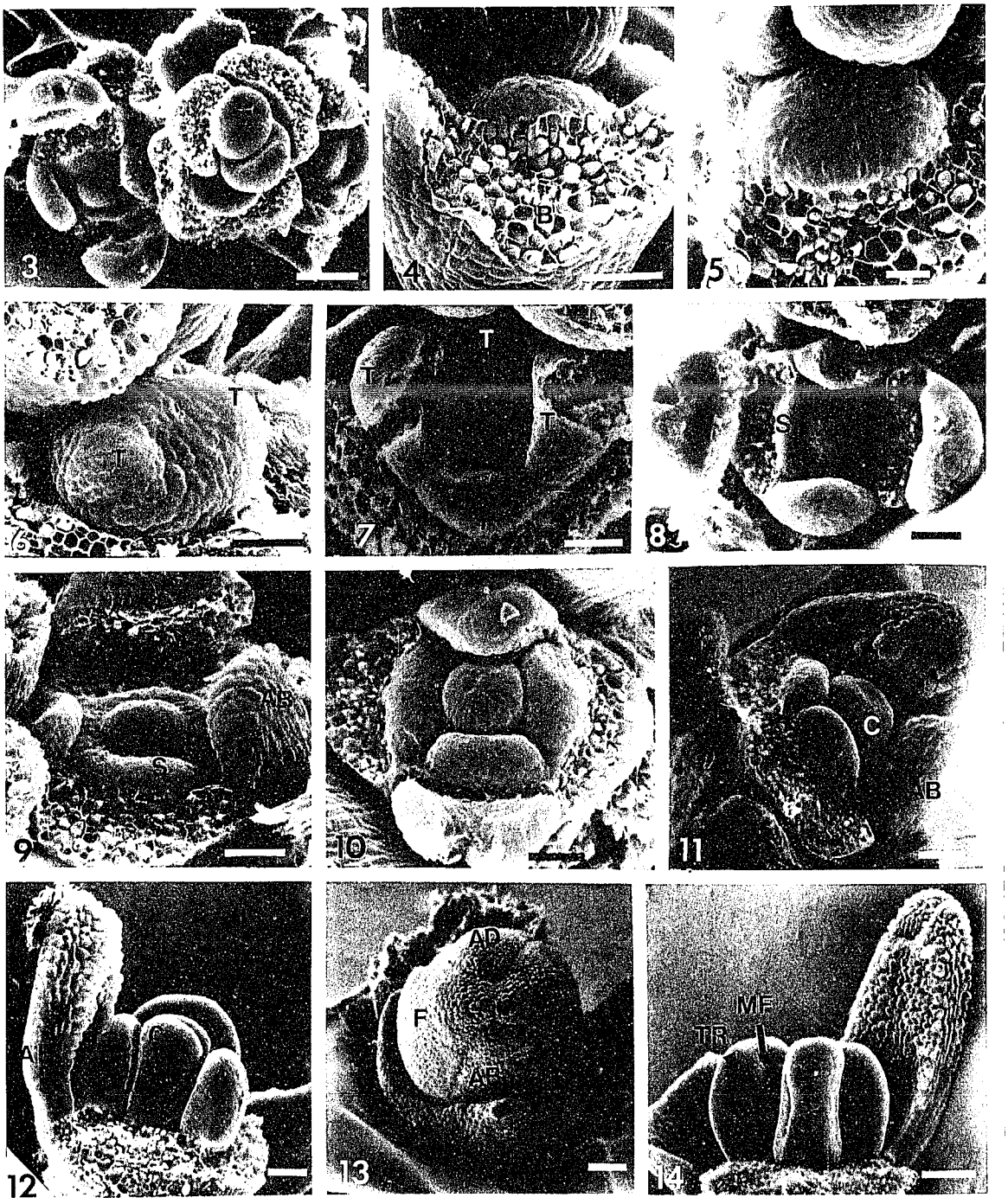


Figs. 3.1-2. Inflorescence and floral drawings of Symphionema montana. **1.** Inflorescence architecture; **2. 2A.** Drawings of open flower. **2B.** Drawing of open flower showing dorsally curved style and anther tube around carpel.

degree at the base of the microsporangia and the distal-most portion of the curving filament (Fig. 3.2, arrowhead in Fig. 3.25). At anthesis, longitudinal dehiscence occurs between the microsporangia of each anther, and the line of dehiscence extends over the frontal distal tip of the anther. No nectaries are present on the floral receptacle. The sessile, glabrous carpel is positioned in the center of the flower, the adaxial suture is present only on the distal quarter of the ovary, although it extends along the narrow abaxially curving style and terminates at the stigmatic area. The stigma is papillate and capitate. The ovary is approximately spheroidal. Two pendulous, orthotropous bitegmic ovules are present (Fig. 3.29) although one is aborted during development.

Organogenesis--Bracts are initiated by the inflorescence apex in a 2/5 anthotaxis (Fig.3). Elongation and enlargement of the internodes between bracts results in sub-opposite organization later in development. Within the axil of a bract, a floral meristem is initiated and is broader frontally than sagittally (approximately 2:1) (Fig. 3.4). The floral meristem continues to enlarge, becoming approximately 160 by 100 μm (Fig. 3.5) prior to the initiation of the first tepal. The first two tepals appear almost simultaneously in the frontal plane (Fig. 3.6). The third tepal initiated is in the abaxial sagittal position, followed rapidly by the initiation of the fourth tepal in the adaxial position (Fig. 3.7). The remaining floral meristem continues to enlarge. Sequential stamen initiation follows the order of the previous whorl of tepals. In antetepalous positions, the two stamens in the median frontal plane are initiated first, one on each side, followed by the succession of the abaxial and adaxial stamen primordia (Figs. 3.8-9). Interestingly, the stamens appear to be raised on a common ring following their initiation (Fig. 3.9). The remaining floral meristem enlarges as a dome and appears to be radially symmetrical (Fig. 3.10). When the remaining floral meristem forms a carpel, it is approximately 85

Figs. 3.3-14. Floral organogenesis and morphogenesis in Symphionema montana. In all figures, the abaxial side of the flower is towards the bottom of image unless otherwise mentioned. **3.** Polar view of inflorescence apex. **4.** A single floral meristem is initiated in the axil of a bract (B, removed). **5.** Polar view of enlarged floral meristem. **6.** Oblique view of floral meristem after initiation of the two lateral tepals (T). **7.** Polar view of floral meristem after all four tepals have been initiated. **8.** Polar view of young flower after initiation of four stamens (S). **9.** Side view of same showing the elevated ring between and beneath the stamens. **10.** Polar view of young flower showing the enlarging carpel primordium (C). **11.** Side view of young flower showing height of carpel and cleft forming. **12.** Side view of young flower (ab=abaxial side). **13.** Polar view of bud showing sagittally-incomplete-valvate aestivation (F=frontal tepal lobes; AD=adaxial). **14.** Adaxially oblique-side view of enlarging flower showing the formation of the median furrow (MF) and the transverse furrows (TR) on the stamen. Scale bars: 3, 13-14 = 100μm; 4-12 = 50μm.



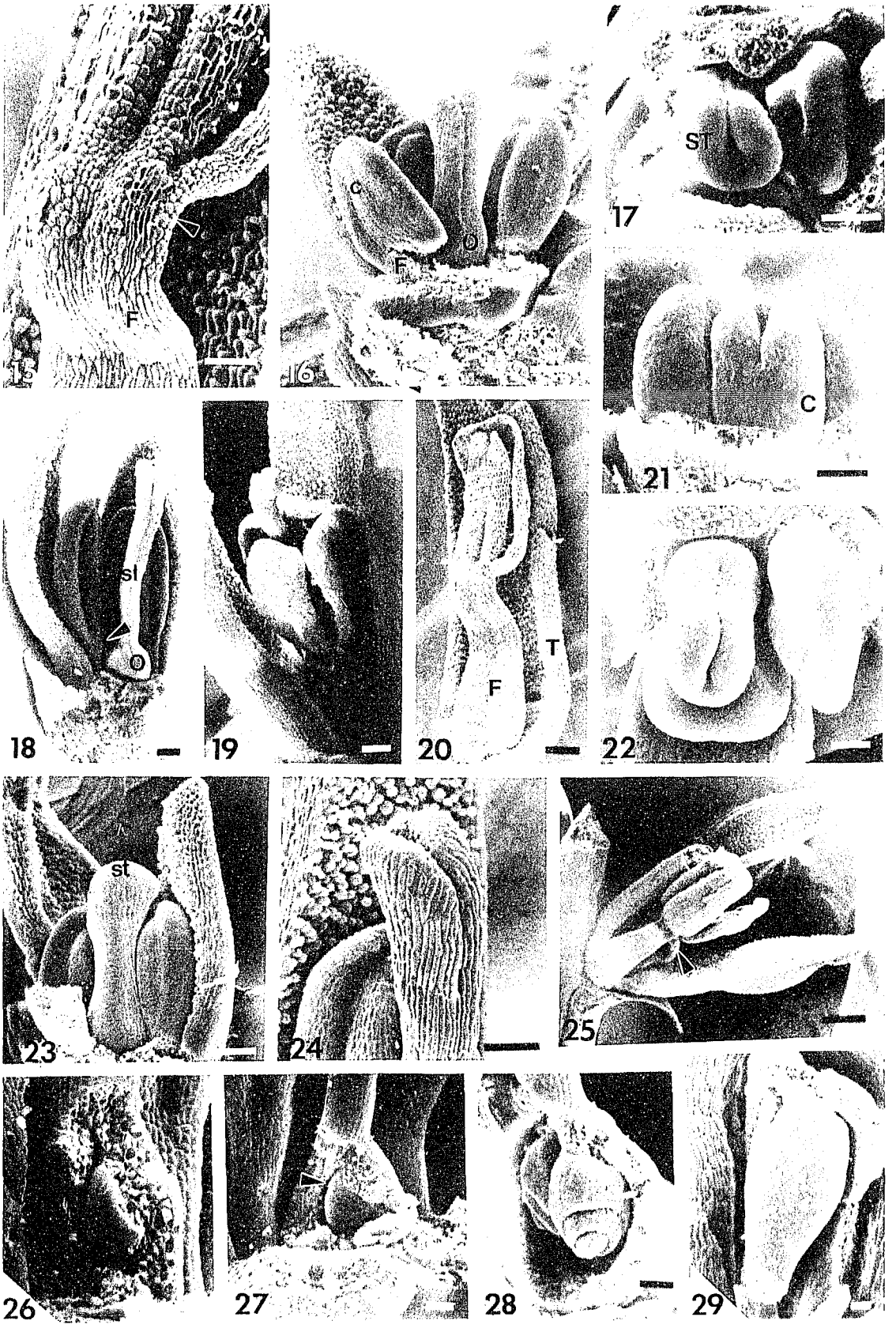
Figs. 3.3-3.14

μm in height. The first signs of cleft formation commence at the top and towards the adaxial or ventral portion on the carpel primordium (Fig. 3.11).

Organ morphogenesis -*Tepals*- - The tepals overtop the inner floral organs and the aestivation pattern is sagittally-incomplete valvate. The epidermis cells of the tepal margins dedifferentiate and become interlocked, beginning at the distal portions of the tepal lobes (Figs. 3.11-12). At aestivation, the tips of the lateral tepals contact one another but those of the adaxial and abaxial tepals do not touch one another (Fig. 3.13). Growth of the tepals is via intercalary growth in the basal half of each tepal, as evidenced by the differentiation of the tepal tips. The distal portions of the tepals curve inward at aestivation, and no Vorläuferspitzen or spines are present (Figs. 3.12-13). Epidermal papillae differentiate on the inner surface of the tepals (Figs. 3.14-15). At anthesis, the papillae become slightly spheroidal in shape in the upper half of the tepal. At anthesis, the tepal lobes separate entirely and reflex approximately 60 degrees in relation to the longitudinal axis of the flower.

Stamens- - Following initiation, the stamen primordia become laterally oblate (Fig. 3.10). Morphogenesis of the thecae begins with the longitudinal crease or median furrow on the adaxial surface (Fig. 3.14). The stamens continue growth and differentiation and at approximately 230 μm stamen height, the transverse furrow forms and the four microsporangia of each stamen are evident. It should be noted that the internal (adaxial) microsporangia are shorter than the outer (abaxial) microsporangia (Fig. 3.14). Slightly later in development, the connective and filament are evident (Fig. 3.16). At the time of thecal differentiation, the transverse furrow of an anther is next to the transverse furrows of the neighbouring anthers (Fig.17). The stamens continue to enlarge and at approximately 700 μm, a slight protuberance develops distally on the filament. The epidermal cells of the protuberences interlock with those of the neighbouring anthers (Fig. 3.15). Neighbouring anthers become post-genitally connate.

Figs. 3.15-29. Late loral morphogenesis of Symphionema montana. **15.** Stamen showing filament (F) curvature and cell dedifferentiation (arrow) at point of connation. **16.** Adaxial view of flower showing the enlarging carpel with ovary (O), the anthers and the short filament. The connective (c) is distinct. **17.** Polar view showing the enlarging stigma (ST). **18.** Adaxial view of older flower showing the ovary (o), style (sl), and stigma. The arrow is pointing to the protrusion of the upper portion of the filament. **19.** Oblique polar view showing anther tube around style. **20.** Removed anther and tepal showing curved filament and anther dehiscence. **21.** Adaxial view of young carpel. **22.** Oblique polar view of older flower. The carpel is elongating and the margins expanding. **23.** Side view of enlarging stigma (st) that tilts abaxially. **24.** Young stigma. **25.** Side view of mature flower with the adaxial and a frontal tepal removed showing the anther tube (arrow at point of connation) and the abaxially recurved style. **26.** Young ovule. **27.** The outer (arrow) and inner integument have been initiated. **28.** Side view of two ovules in locule. **29.** Side view of mature ovule. Scale bars: 15-18, 20, 23-25 = 100 μm; 19 = 200 μm; 21-22, 26-29 = 50 μm.



Figs. 3.15-3.29

At anthesis, the tubular androecium surrounds the style as a result of the post-genital connation (Fig. 3.19). Prior to connation of the filaments, the top of the anther appears to curve inward (arrowhead, Fig. 3.18). The filament becomes slightly bent at the point of connation and narrows slightly from the broad base to the attachment with the anther (Fig. 3.20). Dehiscence of the anthers occurs along longitudinal lines between the thecae and extends to the connective at the base and top of the anther (Fig. 3.20).

Carpel--The cleft of the carpel forms adaxially (Fig. 3.21) and does not extend to the carpel base. The carpel enlarges longitudinally until approximately 180 μm height and the margins expand (Figs. 3.12, 22). Expansion continues at the top of the carpel producing a capitate stigma (Fig. 3.16, 18, 24). When the carpel is approximately 450 μm, the cleft margins of the stigmatic area differentially expand resulting in a slight abaxial curve in the style just proximal to the stigmatic area (arrow Fig. 3.23). The base of the carpel broadens, forming the ovary at a height of approximately 450 μm (Fig. 3.23). Interestingly, the stigma forms before the ovary. Intercalary growth occurs between the ovary and stigma, resulting in the style (compare Figs. 3.23, 18). Papillae begin to differentiate on the inner surface of the stigmatic cleft at approximately 1.3 mm carpel height (arrows in Fig. 3.24). At maturity the capitate stigma curves abaxially (Fig. 3.25).

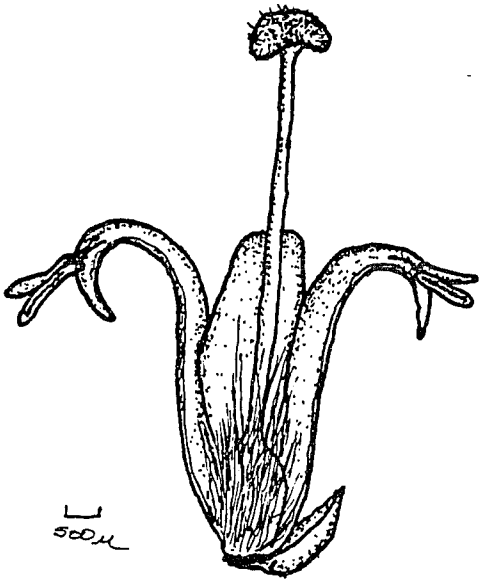
Two ovules are initiated near the top of the locule, when the carpel is approximately 600 μm high (only one ovule shown in Fig. 3.26). The inner integument is initiated first, followed by the proximal initiation of the outer integument (Fig. 3.27). The two ovule primordia are orthotropous (Figs. 3.26-28), By anthesis, one of the ovules appears to be aborted and the fertile ovule is attached by a short funiculus, and the inner integument forms the micropyle (Fig. 3.29).

Stirlingiinae - Stirlingiinae is a unigeneric subtribe in the tribe Conospermeae; the genus Stirlingia includes three species in Western Australia. The plants are perennial shrubs and some have underground lignotubers. One species was investigated developmentally.

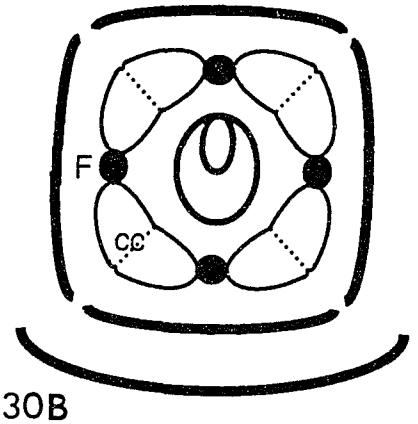
Stirlingia latifolia (R. Br.) Steudel - (Figs. 3.30-48) - Organography - The body of the plant is underground and the leaves are bifacial and lobed resembling those of some Restionaceae (Heywood, 1993). The inflorescence is heterothetic compound, having several secondary inflorescence axes in the axils of lobed to cuneate bracts. Both perfect and functionally male flowers are borne along the terminal, condensed blastotelic racemes, the male flowers tending to be at the base of each inflorescence. The primary inflorescence axis and young shoots are often ensheathed in leaf bases. Twenty to thirty flowers are produced in each secondary or tertiary inflorescence axis; each flower subtended by cuneate bracts.

The yellow to reddish flowers are sessile and at anthesis, the tepal tips are recurved (Fig. 3.30A, 31). The flowers bloom in the early spring but develop in the early summer of the preceding year. Relatively few organogenesis stages were available (collected in September, 1992). The four stamens are adnate to the tepals, with basifixed short filaments. The connate anthers share common locules in bud prior to anthesis (Fig. 3.30B). The stigma is broadly capitate (Fig. 3.46) and held erect by a narrow, straight style. At the stylar base, the spheroidal, hirsute, sessile ovary contains a single basally attached anatropous bitegmic ovule. The fruit is a hairy achene.

Organogenesis - Secondary inflorescence branches develop along the principal axis within the axil of bracts (Fig. 3.32) and sometimes reduced and lobed leaves. Each flowering branch is composed of 12-25 flowers. On each inflorescence branch, the first



30A



30B

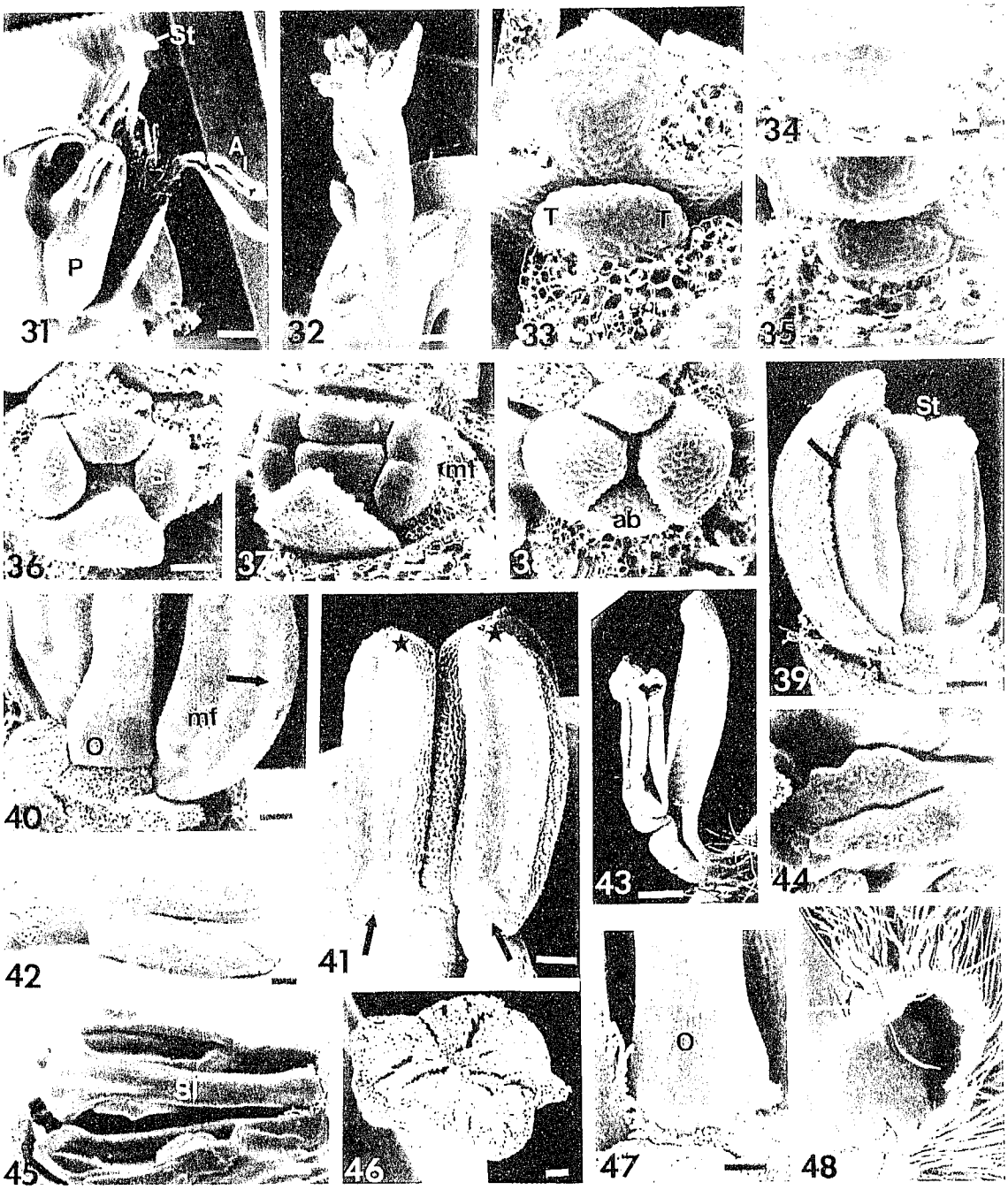
Fig. 3.30. Drawings of Stirlingia latifolia. **30A.** Floral drawing, the lateral tepal has been removed. **30B.** Floral diagram. cc denotes common chambers between neighboring thecae.

two floral bracts are subopposite and are generally smaller than the bracts subtending the inflorescences. The remaining floral bracts are initiated in a low spiral although maintaining a subopposite decussate phyllotaxis (Figs. 3.32). A laterally oblate floral meristem develops (Fig. 3.34; 60 x 30 μm) within the axil of each floral bract. The laterally oblate meristem enlarges (Fig. 3.35) to approximately 110 x 60 μm prior to the initiation of the first two lateral tepals (Fig. 3.33, top flower). The initiation stages of the sagittal tepals and the stamens were not available in the material examined. Following stamen initiation (Fig. 3.36), the remaining floral meristem is approximately 50 x 30 μm . Prior to cleft formation (Fig. 3.37), the meristem enlarges becoming approximately 110 x 70 μm and approximately 100 μm high . The cleft forms adaxially as a rule, but occasionally formed on the lateral side of the carpel primordium (facing a lateral tepal) in some plants. The carpel primordium is broader on the adaxial side than the abaxial side in flowers with the carpel cleft facing the adaxial tepal, (Fig. 3.37).

Organ morphogenesis -*Tepals* - The tepals are sagittally-incomplete-valvate; the tips of the lateral tepals come into contact with one another over the top of the other floral organs. The sagittal tepals fill the corners between the laterals and do not contact one another (Fig. 3.38). It should be noted that the lateral tepals are much broader and longer than the sagittal tepals and the adaxial tepal is longer than the abaxial tepal (Fig. 3.38). The tepals remain glabrous on both the internal and external surfaces (Fig. 3.39). At anthesis, the tepals reflex away from the longitudinal axis of the flower at the point of filament/tepal adnation; the lower portion of each tepal lobe remains interlocked with its neighbouring tepal lobes forming a perigon tube (Fig. 3.31).

Stamens - In the broad stamen primordia, the median furrow develops on the adaxial side, the top, and part of the abaxial side (Fig. 3.37). The enlarging stamen

Figs. 3.31-48. Organogenesis and morphogenesis of flowers of Stirlingia latifolia. **31.** Flower at anthesis with labelled parts: P=perigon; A=anthers; St=stigma. **32.** Young principal inflorescence axis with secondary branches in bract axils. **33.** Inflorescence apex (upper image; IX). Proximal to apex, a floral meristem is present with two tepals in lateral positions (T). **34, 35.** Young floral apex at two stages. **36.** Polar view of young flower showing three stamens (S) and the abaxial tepal (ab). **37.** Oblique polar view of young flower. The median furrow (mf) has formed on the lateral stamens. **38.** Polar view of sagittally-incomplete-valvate aestivation pattern. **39.** Side view of flower. Ridges are present on the anther margins (arrow) and the stigma is enlarging, becoming flared. **40.** Older flower showing the median furrow (mf) on the anther and the ridges (arrow). **41.** Two anthers. The free margins show signs of cell dedifferentiation (arrow) and points of contact with neighbouring filaments. There is a pointed appendage atop each thecae (star). **42.** Abaxial view of stamen, showing the connective and the pointed appendage on each theca. **43.** Side view of mature, dehisced anther. **44.** Polar view of early stigma. **45.** Side view of flower showing the elongate style (sl) and the flaring stigma. **46.** Mature stigma. **47.** Base of carpel. Hairs are being initiated from the receptacle and the base of the ovary (O). **48.** A single, anatropous, basally attached ovule. **Scale bars:** 31-32 = 1 mm; 33, 36-38, 40, 44 = 50μm; 34-35 = 25μm; 39, 42, 45 = 200μm; 41, 46-48 = 100μm; 43 = 500μm.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Figs. 3.31-3.48

primordia are all in contact with one another (Fig. 3.37). A ridge develops at the point of contact between stamens (Fig. 3.39). The 'marginal ridges' surrounding each thecal pair become connate via epidermal modifications (compare arrows in Figs. 3.40, 41). It should be noted that the filaments continue to elongate and become concavely bent proximal to the anthers (Fig. 3.41). There is no appendicular connective (Fig. 3.42). There is zonal growth beneath and between each tepal and its epitepalous stamen (Fig. 3.40). The combination of connation between neighbouring anthers and elongation of the thecae results in an almost alternitepalous position of the anthers, not the filaments (Figs. 3.41, 42; note that the distally conjoined junction of the thecae produces an appendage that resembles a connective appendage of a regular anther, arrow in 41 and 43). At anthesis, the epidermis between neigbouring anther sacs degenerates resulting in common chambers, and dehiscence is via longitudinal lines (Fig. 3.43).

Carpel - There is variation in the orientation of the carpel suture among flowers. In most flowers examined, the cleft faces an adaxial tepal but in some, the cleft forms on the lateral side. Complete and additional developmental materials from other populations are necessary prior to any discussion about the frequency of the lateral orientation. The cleft of the carpel primordium extends across the top, and apparently continues adaxially to the carpel base (Fig. 3.47). The margins expand atop the young carpel, becoming flared (Figs. 3.39, 44, 45). At anthesis, papillae differentiate along the surface of the broad, disc-shaped stigma (Fig. 3.46). An elongate style develops between the stigma and ovary (compare Figs. 3.45, 31). Trichomes develop acropetally on the sessile ovary (Fig. 3.47) and become elongate at anthesis (Fig. 3.48). A single anatropous ovule is present; the placenta and the funiculus are at the base of the ovary (Fig. 3.48). No nectaries develop on the floral base (Fig. 3.47).

Conosperminae - **Conospermum caeruleum** R. Br. (Fig. 3.49-86) -

Organography - Most of the vegetative plant body is underground. There are short internodes separating the leaves that are elongate with an equally elongate petiole. Flowers are borne in condensed racemes. The compound inflorescence is heterothetic and an elongate inflorescence axis terminates the growth of the shoot (anauxotelic). Secondary and in some cases tertiary inflorescence branches are present along the elongate axis. The strongly zygomorphic sessile flowers are borne singly in cuneate bracts. Zygomorphy is expressed in all organs; the perianth arches abaxially. The tepals vary dorsiventrally: the adaxial tepal is broad and at anthesis the distal tip curves adaxially; the lateral tepals are equal in width and the abaxial tepal is broader than the laterals although not as broad as the adaxial tepal (Fig. 3.50). Thetepal bases are densely hirsute. The androecium is zygomorphic; the adaxial anther and the adaxial thecae of the lateral anthers are fertile and the abaxial thecae of the laterals and the abaxial anther are sterile (Fig. 3.49; opposite of Synaphea species). The fertile thecae form a common chamber that holds relatively few extremely large pollen grains (up to 50 μm diameter). The carpel arches adaxially. The style is compressed in bud and the stigma is hooded and porate. The sessile ovary is hirsute with two types of hairs: broadly rounded, elongate hairs on the top and elongate, narrow hairs over the ovary. The species is fly-pollinated and is explosively buzz pollinated, producing a puff of pollen on physical contact with an insect (Johnson and Briggs, 1975; Wrigley and Fagg, 1988).

Organogenesis - Bract primordia (Fig. 3.52) are initiated acropetally on the inflorescence apex flanks in a 2/5 anthotaxis. A laterally oblate floral meristem is initiated in the axil of a bract (Fig. 3.53) that becomes approximately 100 x 80 μm (Fig. 3.54) prior to the initiation of the first tepal. The first two lateral tepals are initiated successively (Fig. 3.55). The sagittal tepals are initiated next, the adaxial tepal before the abaxial tepal (Figs. 3.55, 56). The stamen primordia are initiated in epitepalous

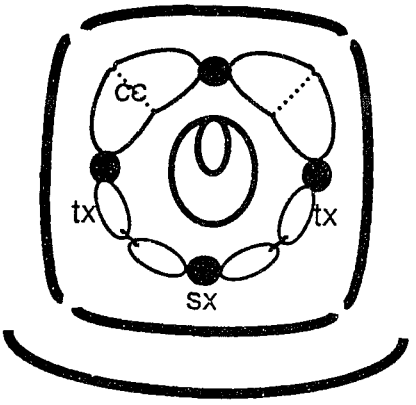
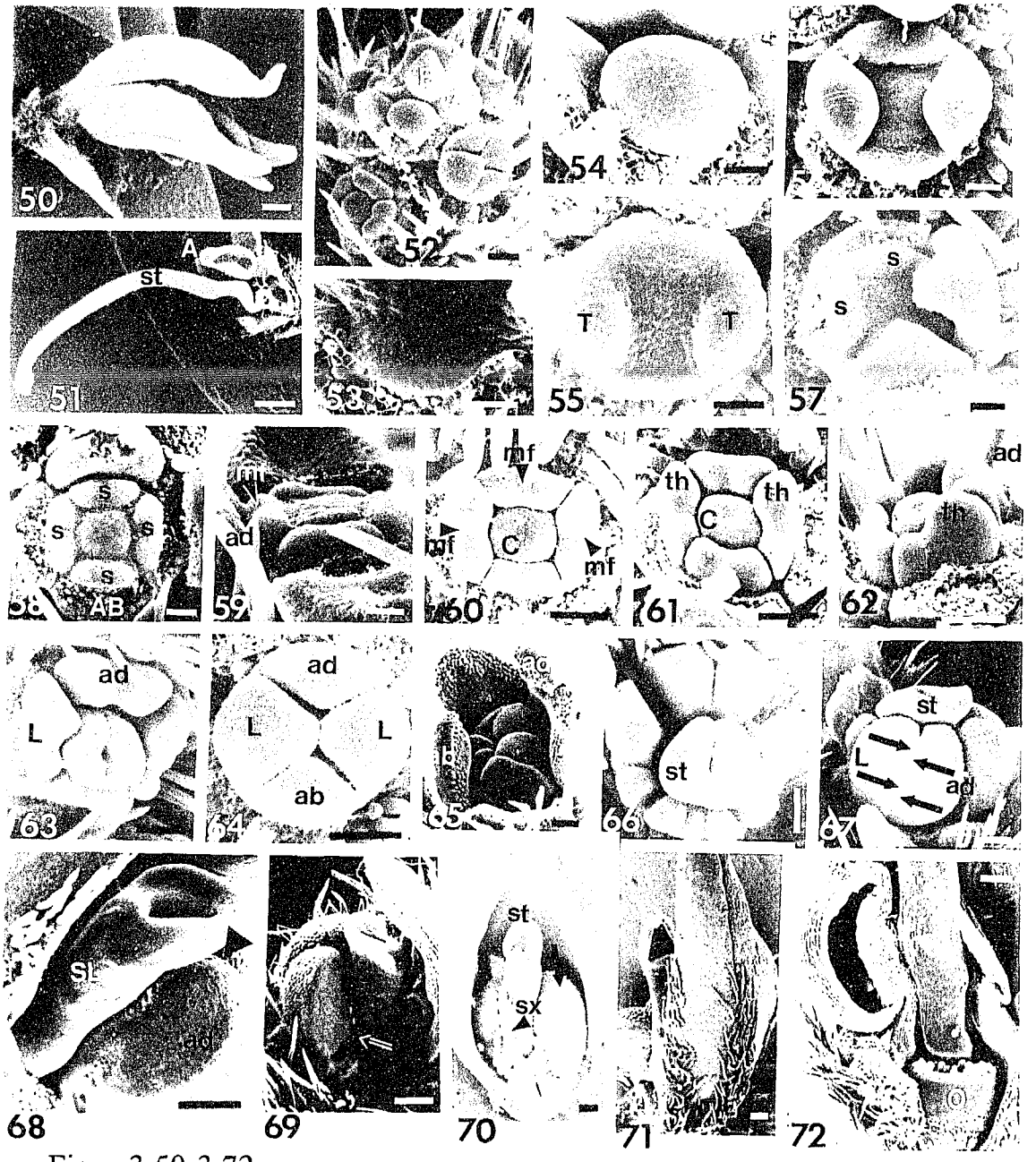


Fig. 3.49. Floral diagram of Conospermum caeruleum. The adaxial anther and the adaxial halves of the lateral anthers are fertile and form common chambers (cc). The abaxial stamen is sterile (sx) as are the abaxial thecae (tx) of the lateral stamen.

Figs. 3.50-72. Organogenesis and morphogenesis of Conospermum caeruleum. **50.** Side view of mature flower at anthesis. B=bract; AD=adaxial tepal. **51.** Mature flower with the tepals removed. The elongate style (st) is compressed proximally. The mature, dehisced anthers (A) are on the adaxial side of the flower. **52.** Side view of inflorescence initiating bract primordia (B). Older flowers are at base. **53.** Polar view of floral meristem in bract axil. **54.** Polar view of enlarged floral meristem. **55.** Polar view of floral primordium with two tepals (T) in lateral positions. **56.** Polar view of floral primordium with all four tepals. **57.** Polar view of floral primordium after stamen (S) initiation. Two of the stamens are covered by the incurving tepal lobes. Two tepals removed. **58.** Polar view of floral primordium showing all four stamen primordia; the abaxial side of the flower is towards the bottom (AB). **59.** Side view of floral primordium. The median furrow (mf) has formed on the stamen primordia. The stamen primordium on the adaxial side (ad) is larger than the others. **60.** Polar view of flower showing the differential enlargement of the stamen primordia (mf=median furrow). **61.** Oblique abaxial view of flower showing the cleft on the carpel (C) and the different sizes of the stamen primordia; the adaxial half of the lateral stamens are larger (th). **62.** Side view of same showing the height differences of stamens and thecae (th) of the lateral stamens. **63.** Oblique polar view of older flower showing the carpel cleft and the different sizes of the thecae and stamens. **64.** Polar view of sagittally-incomplete-valvate aestivation pattern of tepals (ad=adaxial tepal; L=lateral tepals; ab=abaxial tepal). **65.** Side view of partially dissected flower showing different heights of adaxial tepal (ad) to the abaxial tepal (ab). **66.** Polar view of older flower showing the enlargement/flaring of the early stigma (st). The neighboring anthers have been separated to show the margins. **67.** Side view of flower showing the flaring of the stigma (st) and the connate anther margins (arrows). **68.** Oblique view of carpel and adaxial stamen showing the adaxial lip on the stigma (arrowhead) and elongate style (sl). **69.** Side view of enlarging flower. The point of fusion between neighboring filaments is marked by the arrow. **70.** Abaxial view of flower showing the early hooded stigma (st), the elongate staminodia (sx) and the connate thecae between the adaxial anther and lateral anther (arrow). **71.** Abaxial view of flower, the abaxial and right lateral tepal are present. **72.** Side view of enlarging ovary (o). One type of hair is differentiating acropetally on the ovary; another hair type is present on top of the flattened ovary. **Scale bars:** 50-51 = 500μm; 53, 58-59 = 25μm; 52, 54-57, 60-67 = 50μm; 68-72 = 100μm.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Figs. 3.50-3.72

positions and in the same sequence as the tepals, the lateral tepals appearing prior to the sagittal tepals (Fig. 3.57). The remaining floral meristem is laterally broad (60 x 40 μm) and enlarges to a height of approximately 50 μm (70 x 50 μm wide) (Figs. 3.58-60). It is converted into a carpel primordium with continued enlargement and cleft formation (Figs. 3.60-63) beginning as a dimple on the summit (Fig. 3.61). Later, the cleft of the carpel extends down on the adaxial side of the flower (Fig. 3.63).

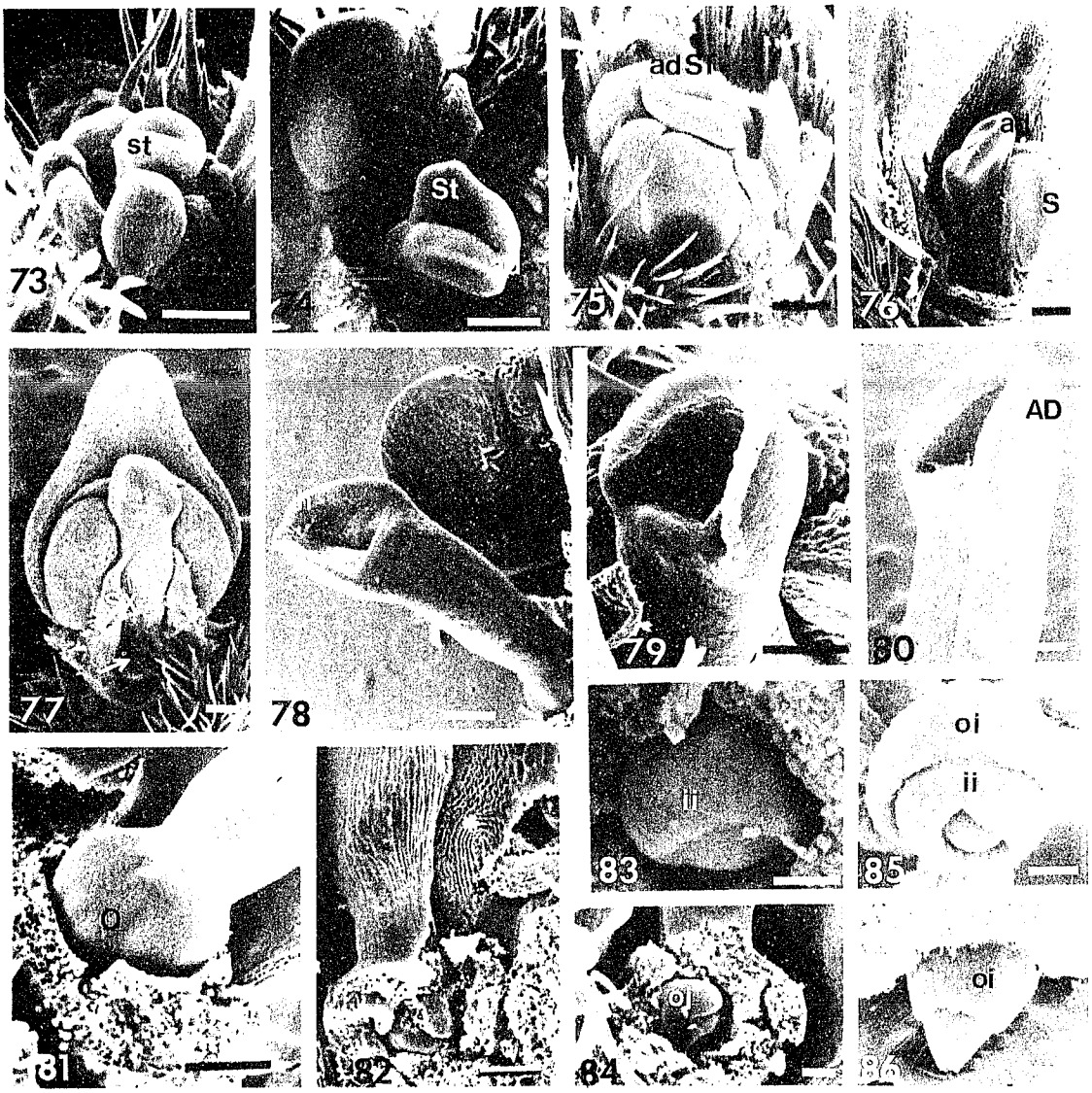
Organ morphogenesis - *Tepals* - The aestivation pattern is sagittally-incomplete valvate, in which the lateral tepals converge over the top of the other floral organs and come into contact with one another (Fig. 3.64). The sagittal tepals converge in the corners between the points of contact of the laterals and do not contact one another. The tepals are glabrous. After anthesis, the adaxial tepal becomes broader than the lateral tepals and the abaxial tepal. Differential elongation and broadening results in the adaxial tepal becoming longer than the abaxial tepal at a tepal height of approximately 500 μm (Fig. 3.65). Elongate hairs develop later at the base of each tepal, next to the valvate tepal margins (Fig. 3.71). The basal portion of each connate tepal lobe (collectively the perigon) becomes densely hirsute at a height of approximately two mm and is narrower than the distal portion (Fig. 3.50).

Stamens - The stamen primordia are different sizes at the earliest stages of development, the adaxial stamens being larger in thickness, width and height (Figs. 3.58, 59). The median furrow develops on the inner, upper and outer sides of the adaxial and lateral stamens, separating the two halves at approximately 15 μm in stamen height (Figs. 3.58-60). The median furrow of the abaxial stamen is later developing than (not as well demarcated) the other stamens (Fig. 3.61). The separate halves (young thecae) of each stamen primordium increase in height differently in each primordium. The adaxial stamen primordium is approximately 60 μm in height, the adaxial half of the

lateral stamens is approximately 60 μm in height, while the abaxial half of the lateral stamens is approximately 40 μm in height and the abaxial stamen is about 30 μm in height (Fig. 3.62). At a slightly later stage in development, the thecae elongate, maintaining a height differential among the different stamens (Fig. 3.63). In all stamens, the thecae overtop the connective (Figs. 3.63 and 66). The dorsiventral symmetry of the androecium becomes more pronounced later in development as the thecae in the adaxial half of the flower become larger in thickness and height (Fig. 3.66). The anthers enlarge while the stamen filament is tightly appressed to the developing style (Fig. 3.68). Zonal growth occurs beneath and between each stamen and tepal resulting in an adnate condition (Fig. 3.69) at a stamen height of approximately 400 μm. The distal most portion of each filament, proximal to the anthers, becomes fused with the neighboring filament (arrows in Figs. 3.69 and 70).

The thecae of different sizes have different developmental pathways and different fates; some are fertile, some sterile. The large thecae, in the adaxial half of the flower, become congenitally fused along their margins (arrow, Fig. 3.67). The fertile thecae have common chambers between neighbouring anthers (Fig. 3.72). The neighbouring epidermal layers (in the common chambers) of the fertile thecae are thin and break away after pollen development (Venkata Rao, 1971). The smaller portions of the lateral anthers on the abaxial side and the smaller, suppressed abaxial stamen primordium have a different developmental course. They elongate late, after being suppressed while the other adaxial fertile thecae develop. Each abaxial half of the lateral anthers and each side of the abaxial anther elongate and become attenuate tapered sterile structures (Figs. 3.70 and 71). The elongate tapered sterile structures are longer on the abaxial anther than on the abaxial side of the lateral anthers (Fig. 3.70). The function of the elongate sterile structures is unknown.

Figs. 3.73-86. Morphogenesis of flowers of Conospermum caeruleum. **73.** Side view of young flower. The young stigma (st) is bilobed and flaring. **74.** Oblique side view of carpel and anther, showing the flaring stigma with differential growth that forms a lip on the adaxial side (ad). **75.** Side view of flower showing the differential growth of the adaxial half of the stigma (ad-ST). **76.** Side view of enlarging carpel. **77.** Abaxial view of flower. The ovule is at the arrow. The sterile halves of the lateral anthers are elongate and tapered (sx). **78.** Oblique abaxial view showing the development of the stigmatic hood (h). **79, 80.** Stigmatic hood nearly mature and at maturity. Note the adaxial side (AD). **81.** Side view of cuboidal ovary (O). **82.** Side view of basal portion of stamen and carpel. The dedifferentiated epidermal cells (*) of the stamen at the point where it is connate with the neighboring stamen (removed). Above the narrow portion, there is adnation (arrow). **83.** Initiation of the inner integument (ii) on the ovule. **84.** Initiation of the outer integument (oi). **85.** Enlarging ovule. **86.** Ovule at maturity. **Scale bars:** 73-82 = 100μm; 83, 85 = 25μm; 84, 86 = 50μm.



Figs. 3.73-3.86

Carpel -- After inception and development of the cleft (Fig. 3.61, 66), the distal portion of the carpel broadens into a clavate structure (Figs. 3.74). The tip begins to form a lip (Fig. 3.67) at a carpel height of approximately 400 μm followed by the expansion of the lateral sides (Fig. 3.74). The marginal expansion signifies the formation of the stigma. Expansive 'marginal' growth of this type continues around to the adaxial side resulting in an expanded clavate stigma that is higher on the adaxial side than on the abaxial side (compare Figs. 3.75, 76). A concave depression forms in the center of the stigma at a carpel height of 600 μm (Fig. 3.77). The adaxial margins continue to elongate, eventually producing a partial hood that overarches the concave entrance to the stylar canal (Fig. 3.78). The abaxial side begins to enlarge at a similar stage (Fig. 3.78) and eventually the combined margins create a hood that is facing the abaxial side of the flower (Fig. 3.70, 79). Prior to anthesis, papillae differentiate inside the rim of the hooded stigma (Fig. 3.80).

The ovary is sessile and broad (Fig. 3.81) with an upward tapered style at approximately 500 μm in carpel height. When the carpel is at a height of approximately 1 mm, the style becomes relatively narrow at the top of the ovary (Fig. 3.81) compared to the more distal portion of the style. The top of the ovary becomes flat (Fig. 3.72) via radial expansion of the sides of the ovary (Fig. 3.81) at a carpel height of approximately 1300 μm. Large rounded hairs develop on the upper portion of the ovary and thinner, tapering hairs are initiated acropetally on the ovary wall (arrow in Fig. 3.72). At anthesis, the style is elongate, and the narrow portion of the style adjacent to the ovary is compressed and sinuous (Fig. 3.51). The epidermis of the style becomes modified and fuses with the anther filaments at the same level as the connation between anthers (asterisk and arrows in Fig. 3.82) The narrow and compressed portion of the style, is below the points of fusion among stamen filaments (Fig. 3.82 arrow in Fig. 3.51).

A single orthotropous ovule is initiated in the upper part of the locule (Fig. 3.77, 83) when the carpel is approximately 500 μm in height. The inner integument forms prior to the outer integument (Figs. 3.84, 85). The ovule is orthotropous and pendulous. At anthesis, the outer integument does not cover the inner integument (Fig. 3.86).

Synaphea polymorpha R. Br. - Organography (Figs. 3.87-122) The plants are shrubs. The main shoot is anauxotelic and each inflorescence axis is a loose terminal blastotelic raceme. Twelve to twenty sessile yellow, extremely zygomorphic flowers are borne in each raceme. Each flower is initiated in the axil of a cuneate bract. Zygomorphy of the flowers is expressed in different organs. At anthesis, the abaxial and lateral tepal lobes reflex partially and differentially. The lateral limbs recurve in relation to the longitudinal axis of the flower. The limb of the adaxial tepal is broad compared to the relatively narrow abaxial tepal limb. In the androecium, the adaxial half of each lateral anther is suppressed in size and sterile, and the adaxial anther is completely sterile and is fused to the base of the broadly capitate, adaxially curved stigma. The fertile thecae of neighbouring anthers are connate. Each stamen has a broad and distally tapering anther that is proximally fused to the base of each tepal. The thecae distally overtop the connective. The broadly spatulate, capitate stigma bears elongate hairs and the style is narrow. The sessile ovary has two types of trichomes: elongate dense hairs cover the ovary and relatively broad, elongate and rounded hairs that are limited to the upper portion of the ovary.

Organogenesis - Floral bracts are initiated on the flanks of inflorescence meristems subopposite ly basally and in a 2/5 anthotaxis distally (Fig. 3.88). A laterally oblate meristem is initiated in each bract axil (Fig. 3.89), becoming approximately 110 x 80 μm (Fig. 3.90) before the first tepals are initiated. The first two tepals are initiated laterally in a successive order followed by the initiation of the abaxial (Fig. 3.91) and lastly the adaxial tepal. Stamen primordia are initiated in a similar

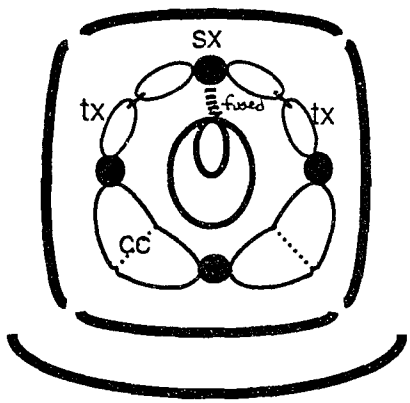
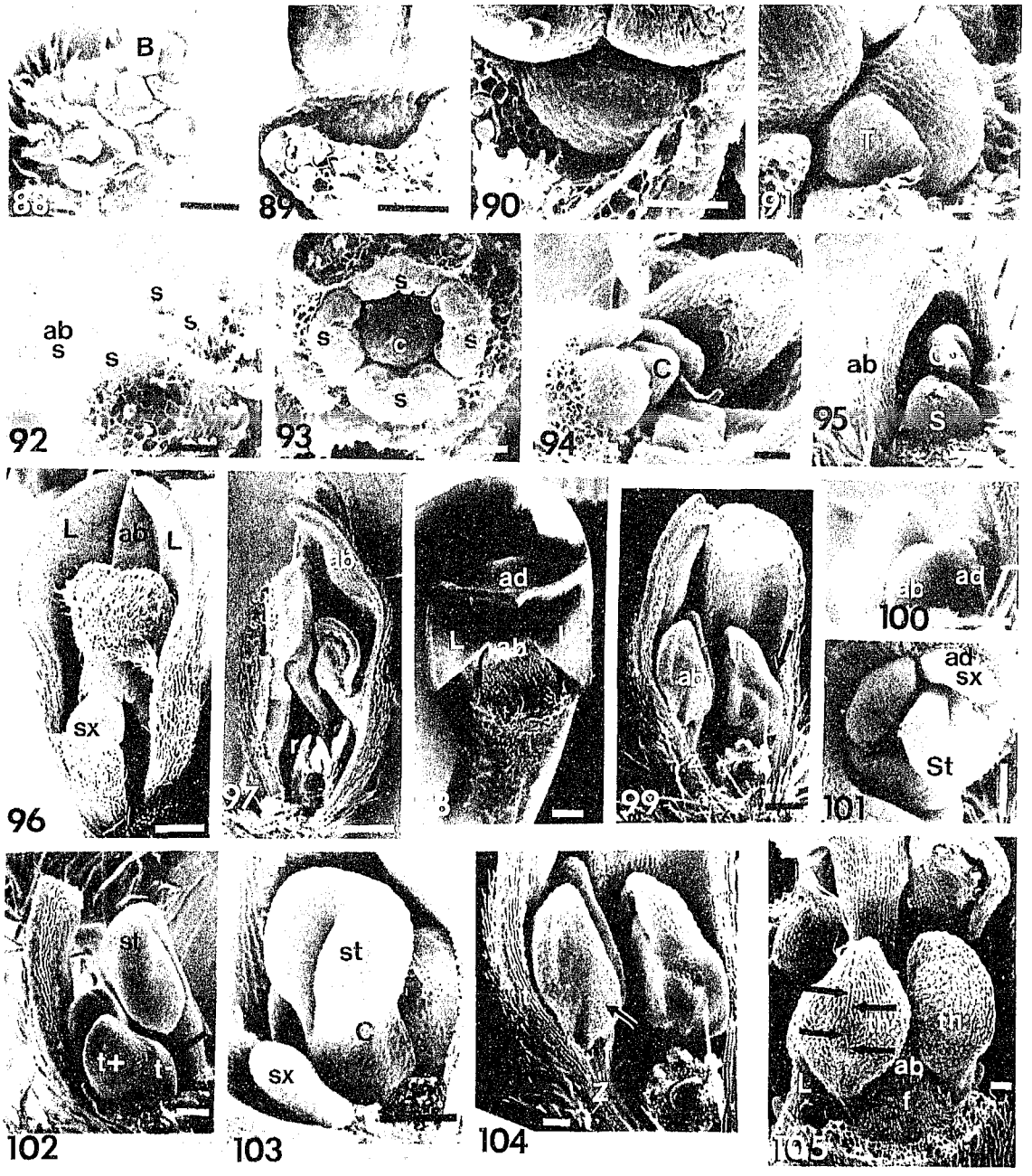


Fig. 3.87. Floral diagram of Synaphea polymorpha. The abaxial stamen and the abaxial halves of the lateral stamens are fertile and form common chambers (cc), opposite to that in Conospermum. The adaxial stamen is sterile (sx) as are the adaxial thecae (tx) of the lateral stamens. F = filament.

Figs. 3.88-105. Organogenesis and morphogenesis in flowers of Synaphea polymorpha. **88.** Side view of young inflorescence initiating bract primordia (B). **89.** Floral apex in bract axil. **90.** Enlarged floral apex. **91.** Side view of floral primordium with two tepals (T) in lateral positions. **92.** Side view of floral primordium after stamen initiation. The abaxial stamen primordium (ab) is larger than the others. The abaxial half of the lateral stamen shown is larger than the adaxial half. **93.** Polar view of same showing the four stamen primordia and the early signs of carpel initiation (c). **94, 95.** Side views of flower showing the enlarging carpel (c). **96.** Preanthesis bud. The adaxial tepal has been removed showing the different sizes of the abaxial (ab) and lateral tepals (L). **97.** Side view of partially dissected preanthesis bud showing the incurved abaxial tepal (ab) and the large rounded hairs on the ovary (r). **98.** Polar view of mature flower showing the arrangement of the tepals. **99.** Side view of flower with the carpel removed. The fertile abaxial stamen (ab) and the fertile abaxial half of the lateral stamen (l) have been separated slightly. **100.** Side view of enlarging flower. The adaxial half (ad) of the lateral stamen is smaller than the abaxial half (ab). **101.** Polar view of early stigma formation (st). The adaxial stamen primordium (sx) will become a staminode. **102.** Side view of flower showing the fertile abaxial half of the lateral stamen (t+)compared to the sterile adaxial half (t-). **103.** Oblique adaxial side view of carpel (c) with an enlarged stigma (st) and a staminode (sx) in the foreground. **104.** Close-up of stamens in Fig. 3.99. Zonal growth (Z) is present between and beneath the filament and tepal. Arrow shows epidermis on stamen that fuses with neighboring stamens (removed). **105.** Abaxial view of stamens. The abaxial stamen (ab) is fertile and its thecae on each side are connate with the neighboring thecae of the lateral anthers (L and arrows). **Scale bars:** 88, 99-103, 105 = 100μm; 89-95, 104 = 50μm; 96-98 = 500μm.



Figs. 3.87-3.105

sequence. The remaining floral meristem is 90 x 80 μm and enlarges to approximately 120 x 110 μm during carpel inception. The carpel cleft is formed on the adaxial side (Fig. 3.94).

Organ morphogenesis - *Tepals* - The aestivation pattern is sagittally-incomplete valvate. Trichomes develop abaxially on each tepal (Fig. 3.95). Differential broadening occurs in tepals at a tepal height of approximately 1 mm. The abaxial tepal does not appear to broaden (Fig. 3.96) but its upper portion arches inward (Fig. 3.97). The upper half of the lateral tepals, above the point of tepal-filament adnation, becomes broad. The margins of the lateral tepals appressed to the adaxial tepal appear to curve inward in relation to their longitudinal axis (compare Figs. 3.96 and 98). Trichomes do not develop on the upper portions of each tepal or are ephemeral. At anthesis, the lobes bend downwards, the lateral lobes and abaxial lobe forming a trilobed lip, and the adaxial lobe appearing as a flat to concave hood (Fig. 3.98).

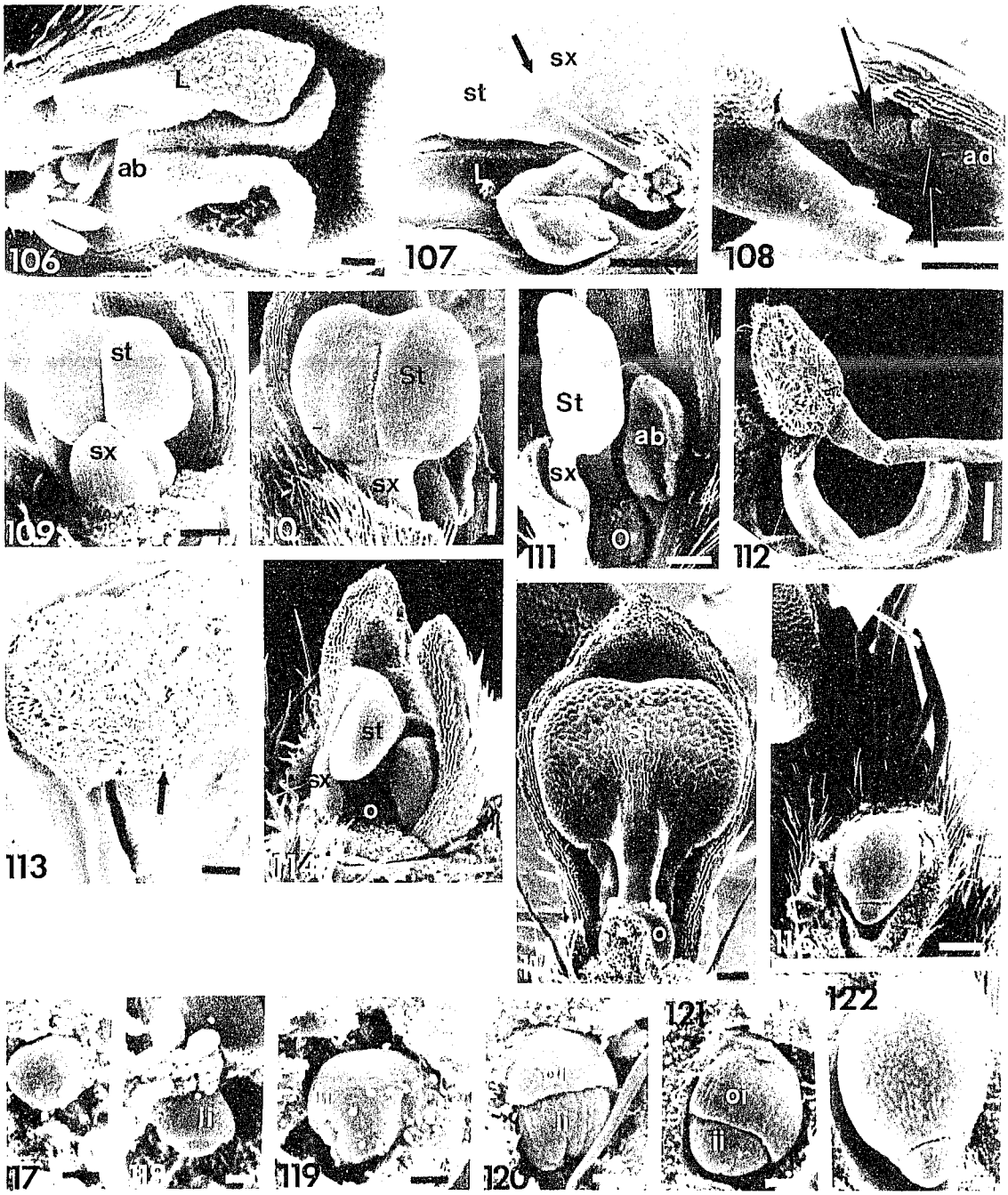
Stamens - The abaxial stamen primordium appears larger than the lateral primordia, that are larger than the adaxial primordium (Figs. 3.92, 93). The median furrow becomes visible on the inner face, top and part of the back of the lateral and abaxial primordia (Figs. 3.100, 101), although poorly defined in the latter. The lateral sides of the differentiating abaxial stamen primordium and abaxial thecae of the lateral anther primordia are in contact with one another (Fig. 3.101). Following the development of the transverse furrow, a ridge develops on the margins in contact between the abaxial anther and the abaxial thecae of the lateral anthers (Fig. 3.102). The adaxial thecae on the lateral anthers do not develop (arrows in Figs. 3.99, 102). The adaxial anther primordium develops as a rounded laminar staminodium (Figs. 3.100, 103). Zonal growth has begun beneath and between each tepal and its epitepalous stamen (Figs. 3.marked 'z' and arrow in 99, 104). The 'marginal ridges' surrounding

each fertile thecal pair, from different anthers, become connate via epidermal modification (arrows in Fig. 3.104). There is no appendicular connective on the abaxial anther (Fig. 3.105). Two lobes form on each tepal at the point of filament/tepal adnation (Figs. 3.107, arrow in 108). The androecium become connate at the base of the filament (Figs. 3.107, 108). Each anther sac (Figs. 3.96, 104) is covered by a thin epidermis (Venkata Rao, 1971). Prior to anthesis, the epidermis between neigbouring anther sacs gives way so common chambers result (Fig. 3.106). At anthesis the connate portions separate, allowing pollen to be shaken out by pollinators. The anthers are enclosed by the perianth tube through anthesis, an uncommon feature among flowers in genera of Proteaceae (see Placospermum and Conospermum).

The adaxial staminodium has a separate developmental pathway after initiation. The adaxial staminodium appears laminar (Fig. 3.103). The distal portion of the staminodium comes into contact with the basal portion of the differentiating stigma (Fig. 3.109). The top of the staminodium becomes fused to the base of the young stigma (Figs. 3.110, 111). The two structures remain fused through anthesis (Fig. 3.112).

Carpel - The cleft of the young carpel (Fig. 3.101) does not extend to the floral base (Figs. 3.101, 103). Stigma formation begins when the top margins of the carpel broaden, resulting in a clavate or bifid structure (Fig. 3.100, 101). Expansion of the young stigma continues (Fig. 3.103). Papillae differentiate centripetally from the center of the stigma (Figs. 3.110). The stigmatic surface is densely hairy at maturity (Fig. 3.113). Intercalary growth between the ovary and the clavate stigmatic area results in an elongate style, (Fig. 3.114). The ovary enlarges radially after the style is delineated (Fig. 3.114). Broadly rounded trichomes begin to develop on the top of the ovary (Fig. 3.115) followed by the differentiation of smaller trichomes along the sides of the ovary. Both types of trichomes elongate (Fig. 3.116). A single ovule is initiated at the top of the loculus from a submarginal position (Fig. 3.117). The inner integument is initiated prior

Figs. 3.106-122. Morphogenesis of flowers of Synaphea polymorpha. **106.** Side view of connate anthers: ab=abaxial anther; l=lateral anther. **107.** Side view of older flower showing lateral tepal (L) and and the fused stigma (st) and staminode (sx). **108.** Adaxial tepal (ad) and the lobes on the filament (arrows). **109.** Adaxial view of flower showing the contact between the staminode (sx) and the base of the young stigma (st). **110.** Later stage showing the point of fusion between the staminode (sx) and the base of the stigma (st). Papillae are differentiating on the stigma. **111.** Side view of flower showing fusion between stigma and staminode. The fertile abaxial anther (ab). Large rounded hairs are differentiating on the top of the ovary (o). **112.** Dissected carpel (top) and staminode (bottom) at anthesis. **113.** Mature stigma; the staminode has been removed (arrow). **114.** Side view of flower showing the staminode (sx) on the adaxial side, the ovaary and the broadening stigma (st), the fertile abaxial anther (ab) and the abaxial tepal (T). **115.** Abaxial view of older carpel showing the back of the stigma (st) and hairs developing on top of the ovary (o). **116.** Side view of dissected ovary showing the large round hairs (in black) atop the ovary and the thin, narrow hairs around the ovary. Ovule at the base. **117.** Young ovule. **118.** Inner integument (ii) initiated on ovule. **119.** Enlargement of the inner integument (ii). **120.** Outer integument (oi) initiated. **121.** Enlargement of integuments. **122.** Mature ovule. **Scale bars:** 106, 109, 114-116 = 100μm; 107, 112 = 500μm; 108, 110-111, 113 = 200μm; 117-122 = 50μm.



Figs. 3.106-3.122

to the outer integument (compare Figs. 3.118-120). The outer integument does not overtop the inner integument (Figs. 3.121, 122).

Petrophilinae - Petrophilinae includes two genera, Petrophile (42 species) and Isopogon (34 species) both of which are common in Western Australia, although several species are found in eastern Australia (Johnson and Briggs, 1975; Wrigley and Fagg, 1988). The subtribe is recognized based on the presence of numerous flowers in condensed heads, and swollen styles that are often hairy. The swollen distal portions of the style have been termed pollen presenters as the pollen is deposited from the anthers to the pollen presenter at anthesis. The genera are divided primarily on the persistence of the floral bracts: persistent in Petrophile and ephemeral in Isopogon (Venkata Rao, 1971; Johnson and Briggs, 1975). The plants are generally woody shrubs that are highly adapted to xerophytic environments. The flowers are functionally actinomorphic, the upper portions of all four tepals reflexing equally. Most of the taxa also have a true perianth tube, that develops via intercalary growth between and beneath each of four tepal lobes (Venkata Rao, 1967; 1971). Like most Cennarhenieae, the flowers lack nectariferous glands.

Development was compared among three species of Isopogon (I. formosus R. Br., I. cuneatus R. Br., I. anemonifolius (Salisb.) Knight), and Petrophile serruriae R. Br. The floral and inflorescence ontogeny of Isopogon formosus is described as typical of the subtribe. Early and late floral ontogeny are very similar among all examined taxa although there were differences in the mature condition of the pollen-presenters and anthers. In addition, Petrophile serruriae has rounded spines called Vorläuferspitzen on the tips of the tepals (Baum, 1951).

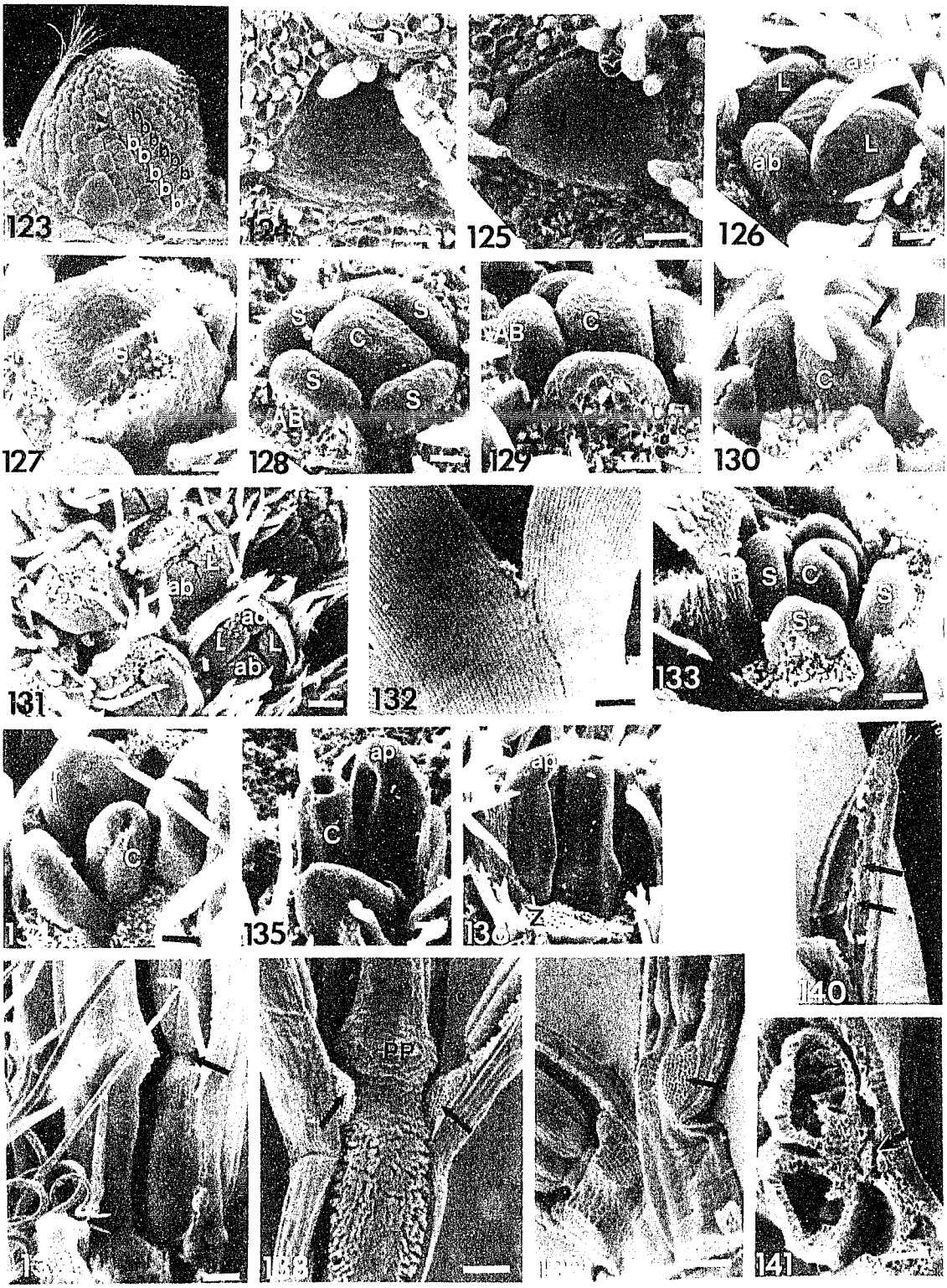
Isopogon formosus (Figs. 3.123-156) - Numerous pinkish red flowers are borne in terminal head-like inflorescences, reaching anthesis in acropetal sequence. The condensed heads resemble Asteraceae and are commonly referred to as "cone-flowers."

Organogenesis - The inflorescence meristem initiates bract primordia in a high anthotactic spiral (Fig. 3.123). Within the axil of each bract, a floral meristem is initiated, becoming approximately 125 μm wide (Figs. 3.124, 125) prior to organogenesis. The lateral tepals appear to be initiated prior to the sagittal tepals (Fig. 3.126). The stamens are initiated in a similar sequence as the tepals, in epitepalous positions (Fig. 3.127). The remaining floral meristem enlarges to a width of approximately 90 μm and a sagittal-diameter of 60 μm (Figs. 3.128, 129) prior to the inception of the carpel. The cleft forms adaxially on the carpel primordium (Fig. 3.130).

Organ morphogenesis -*Tepals* - The pattern of aestivation is sagittally-incomplete valvate (Fig. 3.131). Trichomes develop abaxially on the arched tepals (Fig. 3.131) in the same sequence they were initiated. The trichomes become elongate and do not develop on the lower portions of the tepals (Fig. 3.131, 140). Zonal growth appears to begin beneath and between the tepals, resulting in the development of a fused perianth tube (Fig. 3.132) at a height of approximately 1.5 mm.

Stamens - The stamen primordia enlarge to a height of approximately 100 μm before the median furrow demarcates the thecae (Fig. 3.133). The median furrow extends down the central face of each stamen primordium. The transverse furrow becomes demarcated when the stamen primordia are approximately 200 μm high (Fig. 3.134). A distinct connective appendage begins to form terminally later in development (Fig. 3.135). The filament becomes noticeable as does the zonal growth beneath and between each tepal and anther (arrow in Fig. 3.136). The thecae of each anther are appressed to neighboring anthers. The appendicular connective seems to arch over the

Figs 123-141. Organogenesis and morphogenesis of flowers of Isopogon formosus. **123.** Young inflorescence apex initiating bract primordia (b). **124.** Floral meristem in bract axil. **125.** Enlarged floral meristem. **126.** Oblique side view of floral primordium after tepal initiation (ad=adaxial tepal; ab=abaxial tepal; L=lateral tepals). **127.** Side view of floral primordium after stamen initiation. **128.** Oblique polar view of early carpel (c) development. **129.** Side view of flower showing height of carpel. **130.** Side view of flower showing the formation of the cleft on the adaxial side (arrow). **131.** Polar view of inflorescence showing the sagittally-incomplete-valvate aestivation patterns of the tepals. **132.** Close-up of tepals where zonal growth has resulted in a tube. **133.** Side view of flower showing the heights of the stamens (s) and the carpel (c). **134.** Adaxial polar view of young flower showing the formation of the transverse furrow (tr) on the stamens and the porate terminus of the carpel (c). **135.** Side view of later stage showing anther connective appendage (ap) and the porate carpel. **136.** Side view of later stage showing the appendicular connective appendage overtopping the carpel and zonal growth (z) between the tepal and filament. **137.** Midregion of older flower showing the lobe between the filament/tepal insertion and the short filament (arrow). **138.** Midregion of older flower showing the increased size of the lobe. The lobes surround the constricted pollen-presenter (pp). **139.** Epidermal cells of the lobes at the point of tepal/filament adnation. **140.** Removed tepal. The anther has been removed and the line of fusion between the connective and the tepal is present (arrows). **141.** Cross section of older anther, before anthesis, showing the point of fusion between the connective and the tepal (arrow). **Scale bars:** 123 = 500μm; 124-130 = 25μm; 131-132, 137, 141 =100μm; 133-136 = 50μm; 138-140 = 200μm.

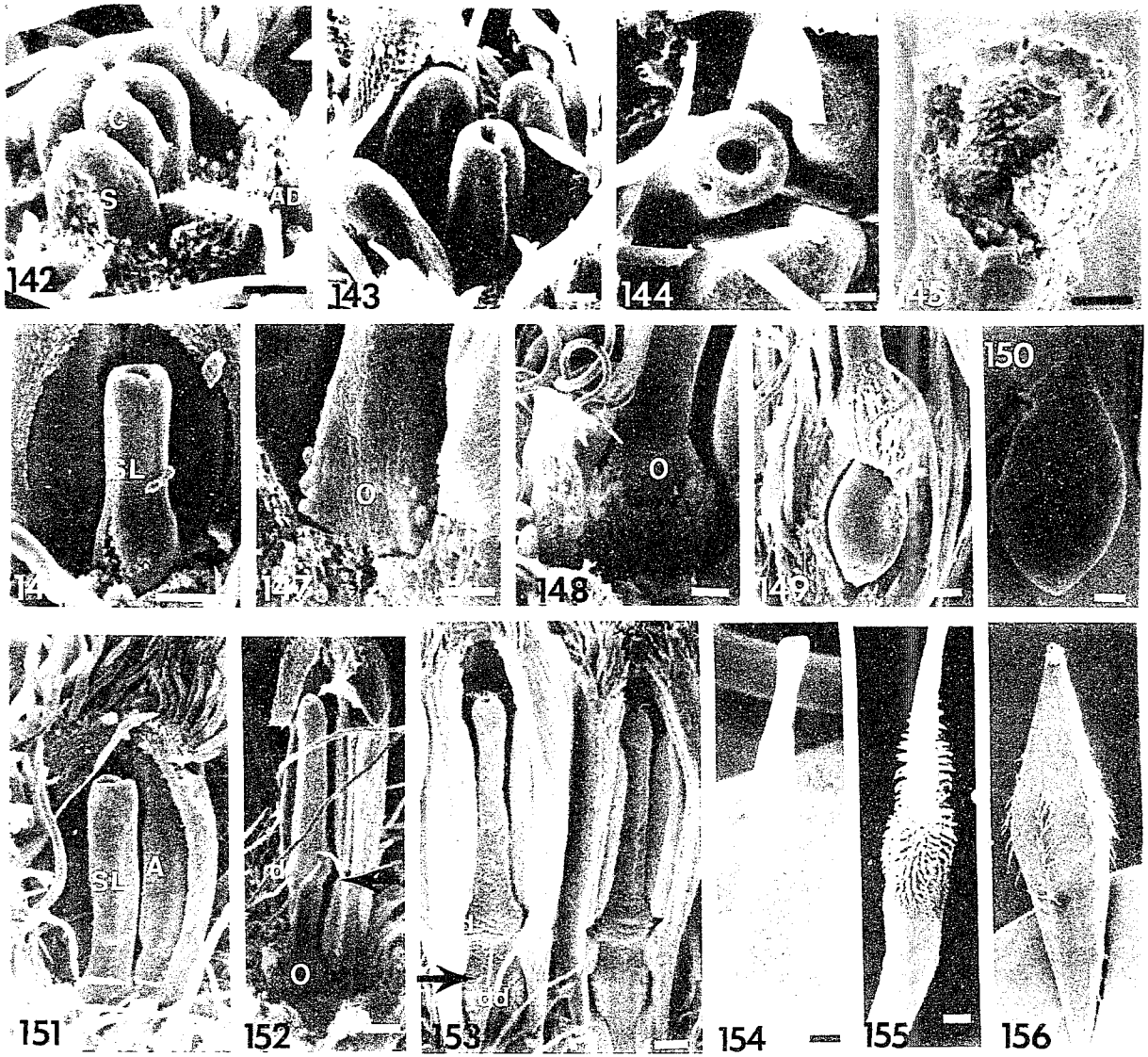


Figs. 3.123-3.141

developing carpel at the same stage (Fig. 3.136). As zonal growth lifts the anther above the floral receptacle, the point of adnation between the filament and tepal becomes lobed (Fig. 3.137). The lobes enlarge and become fused to the neighbouring tepal/filament lobes (arrows in Figs. 3.138, 139). The **abaxial** side of each anther **connective** fuses with the adaxial surface of the tepal at a height of approximately 2 mm (Fig. 3.140, anther removed, Fig. 3.141, section of older flower). It should be noted that this is the only group of proteaceous taxa where the connective of the anther becomes fused to the tepal based on current observations. Adnation of the anther connective to the tepal is similarly present in Petrophile. Prior to anthesis, the anthers dehisce along longitudinal lines, depositing their pollen on the exserted pollen presenter.

Carpel -- The carpel cleft does not extend to the carpel base (Fig. 3.142). The carpel enlarges and increases in height, growing as a tubular structure and maintaining a open cleft adaxially and distally (Fig. 3.143). The margins fuse leaving a terminal pore (Fig. 3.144). The carpel continues to enlarge and the stigmatic area remains porate. Scattered papillae differentiate on the inner surface of the porate stigma prior to anthesis (Fig. 3.145). The ovary is sessile at 280 μm height (Fig. 3.146). The ovary becomes cuboidal (Fig. 3.147) and hairs differentiate acropetally from the base along the rounded corners (Fig. 3.147). At a later stage, hairs are initiated and begin to develop between the corners (Fig. 3.148). From the base of the ovary and the receptacle, elongate thick hairs develop contrasting with the short and thin ovarian hairs (Fig. 3.149). The single bitegmic ovule is at first orthotropous and is initiated in the upper part of the loculus (Fig. 3.149). There is enlargement of the ovule that produces a tapered structure on the side opposite of the micropyle (Fig. 3.150). The ovule is hemitropous at maturity (Fig. 3.150).

Figs. 3.142-156. Floral morphogenesis in Isopogon formosus. **142.** Side view of flower showing the extent of the cleft on the adaxial side of the flower. **143.** Side view of flower with a porate stigma. **144.** Polar view of carpel terminus with porate tip. **145.** Split stigma at maturity. **146.** Side view of enlarging carpel. The style (sl) is elongating and the ovary is broadening. **147.** Development of trichomes on ovary. **148.** Later stage of trichome development on ovary. **149.** Side view of sectioned ovary showing ovule, narrow hairs around the top and the broader and longer hairs from the base of the ovary. **150.** Mature hemitropous ovule. **151.** Side view of flower showing the elongate style (sl) next to the anther (A). **152.** Side view of larger carpel showing the development of the first disk (d) at the midregion of the pollen-presenter. **153.** Side view of two flowers showing the presence of a second disk (dd). **154.** Mature pollen-presenter of Isopogon formosus. **155.** Mature pollen-presenter of Isopogon cuneatus. **156.** Mature pollen-presenter of Petrophile serrurieae. **Scale bars:** 142-147, 149 = 50μm. 148, 150-151 = 100; 152-156 = 200μm.



Figs. 3.142-3.156

Pollen presenter and style -- At maturity, flowers of Petrophilinae (Figs. 3.154-156) have a secondary pollen presentation system or a pollen presenter. That is, a modified structure on the upper portion of the style, basal to the stigma, holds the pollen from the same flower at anthesis. The dehisced anthers reflex away from the pollen presenter at anthesis. Pollinators foraging the flowers will come into contact with the pollen on the pollen presenters and deposit pollen on the stigma from other plants.

In Isopogon, the carpel continues to elongate. Differential broadening occurs at different heights so that two disks form. At about one mm carpel height, a disk-like lobe broadens around the developing style at a height of approximately 450 μm above the base of the carpel, adjacent to the base of the anthers (arrows in Fig. 3.152). Expansive radial growth of the carpel forms a second disk (arrow Fig. 3.153), proximal to the first disk, beginning at a carpel height of approximately 1500 μm concomitant with the time that the filament/tepal lobes are becoming fused. The disks becomes lobed in appearance at a slightly later stage, the lobes being positioned opposite the neighbouring tepal/anther margins (Fig. 3.138). Hairs develop on the lobes. The point of tepal/anther lobe fusion appears to constrict the enlarging pollen presenter and separates the two disks (Fig. 3.152). Short hairs are developing on and between the pollen presenter lobes proximal to the constriction (arrows Fig. 3.152). The pollen presenter has radially enlarged distal to the constriction (Fig. 3.152). The short hairs have differentiated basipetally from the original disk and lobes (Fig. 3.153) later in development. At maturity, the pollen presenter is villose and is positioned approximately one mm from the porate stigma (Fig. 3.154). The pollen presenter of Isopogon cuneatus is illustrated (Fig. 3.155). The major difference to other taxa is the erect rows of hairs which extend down the pollen presenter (Fig. 3.155). The pollen presenter of Petrophile serruriea is shorter than those in the Isopogon species examined but is similarly wooly (Fig. 3.156).

Franklandieae - Adenanthiinae - Adenanthos Labill. is commonly referred to as Wooly Bush or Jug Flower. Species are woody perennials, small trees, shrubs or undershrubs, with single stems from root-stocks or multiple stems from lignotubers. Leaf forms vary between simple and entire, lobed, deeply lobed, terete and, filiform or segmented, most of which have a gland on the distal tip (Nelson, 1978). Its taxonomic position within Franklandieae is shared with the unique genus Franklandia (Haber 1959, Johnson and Briggs 1975) although Nelson (1978) calls it a dubious relationship.

Adenanthos is morphologically isolated from other Australian Proteoideae (Johnson and Briggs, 1975). One of the most distinguishing characters of Adenanthos is the presence of a single flower within an involucre of scale-like or imbricate bracts. The sessile flowers are bisexual, strongly protandrous, and morphologically diverse (Nelson, 1978). Symmetry of the flowers varies between slight zygomorphy and extreme zygomorphy (Nelson, 1978). The four tepals are connate into a tube. The basifixed anthers are adnate to the tepals via a very short and often basally lobed filament . At anthesis, the distalmost portion of each tepal reflexes away from the longitudinal axis of the flower, where the short filaments are attached.

Nelson's (1978) revision of the genus included 32 species in two sections. There are variations between the stamens of the two sections (Nelson, 1978). Flowers in taxa of Section Eurylaema (three species) have a sterile abaxial stamen (staminodia) whereas flowers in the section Stenolaema (Adenanthos sensu Nelson) have four fertile stamens. The anthers dehisce prior to anthesis, depositing pollen on a modified stylar structure termed a pollen-presenter. The pollen-presenter morphology varies between the sections: sagittally flattened (spatulate) in sect. Eurylaema and actinomorphic or vertically grooved in sect. Stenolaema.

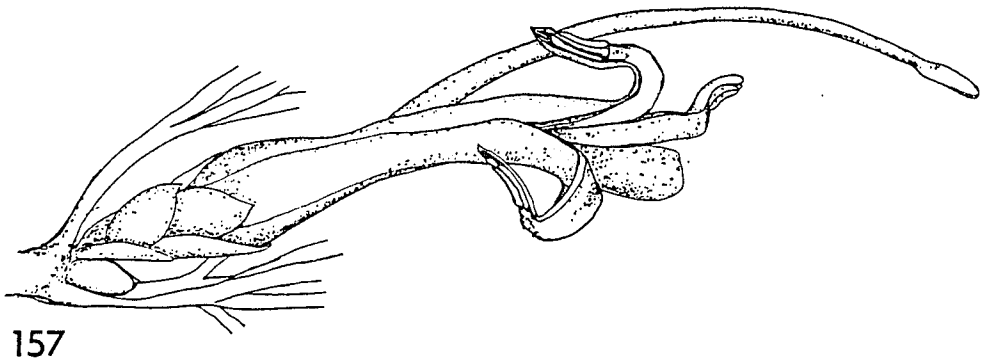
The glabrous or hirsute gynoecium is sessile, surrounded by four hypogynous scales (nectaries) and a ring of long hairs. The ovary contains a single, laterally attached, hemitropous, bitegmic ovule. The arcuate style is longer than the tepals in most cases, emerging between the adaxial and one of the lateral tepals prior to anthesis. The pollen presenter, the stigma and the upper portion of the style are held between the four anthers prior to anthesis. The stigma is a bifid slit extending proximally along the adaxial side of the pollen-presenter. The stigmatic groove is internally papillate and opens at receptivity.

Development was compared among three species of Adenanthos: two species from section Stenolaema, A. oreophilus Nelson and A. sericeus Labill., and one species from section Eurylaema, A. obovata Labill. The floral ontogeny of A. oreophilus and A. obovata is described.

Section Stenolaema (Adenantos sensu Nelson, 1978) -

Adenanthos oreophilus (Figs. 3.157-186) - A. oreophilus occurs in southwestern Australia. The shrubs are densely wooly, and the leaves are terete and segmented (Nelson, 1978). The inflorescence appears to be terminal (anauxotelic) although additional material needs to be examined. Five to seven bracts are borne on each inflorescence and a single flower develops in one of the axils.

The zygomorphic flowers are reddish-orange with villous perianth lobes. The top portion of each lobe (limb) is free at anthesis and recurves from the longitudinal axis of the flower just below the point of filament/tepal adnation (Figs. 3.157, 158). The perianth tube is formed by the connation of the tepal lobe margins. The tube is longer on the abaxial side as the arched style is exserted from between the adaxial and a lateral tepal lobe. The style arches abaxially in relation to the subtending floral bract. The pollen presenter is sagittally broad and limited to the upper region of the style and



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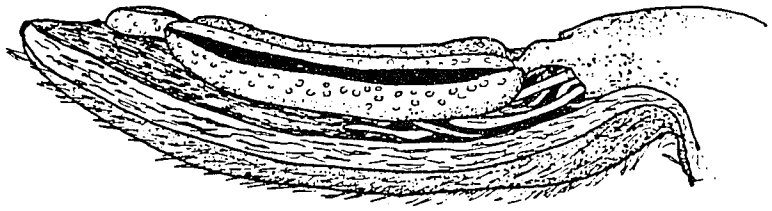

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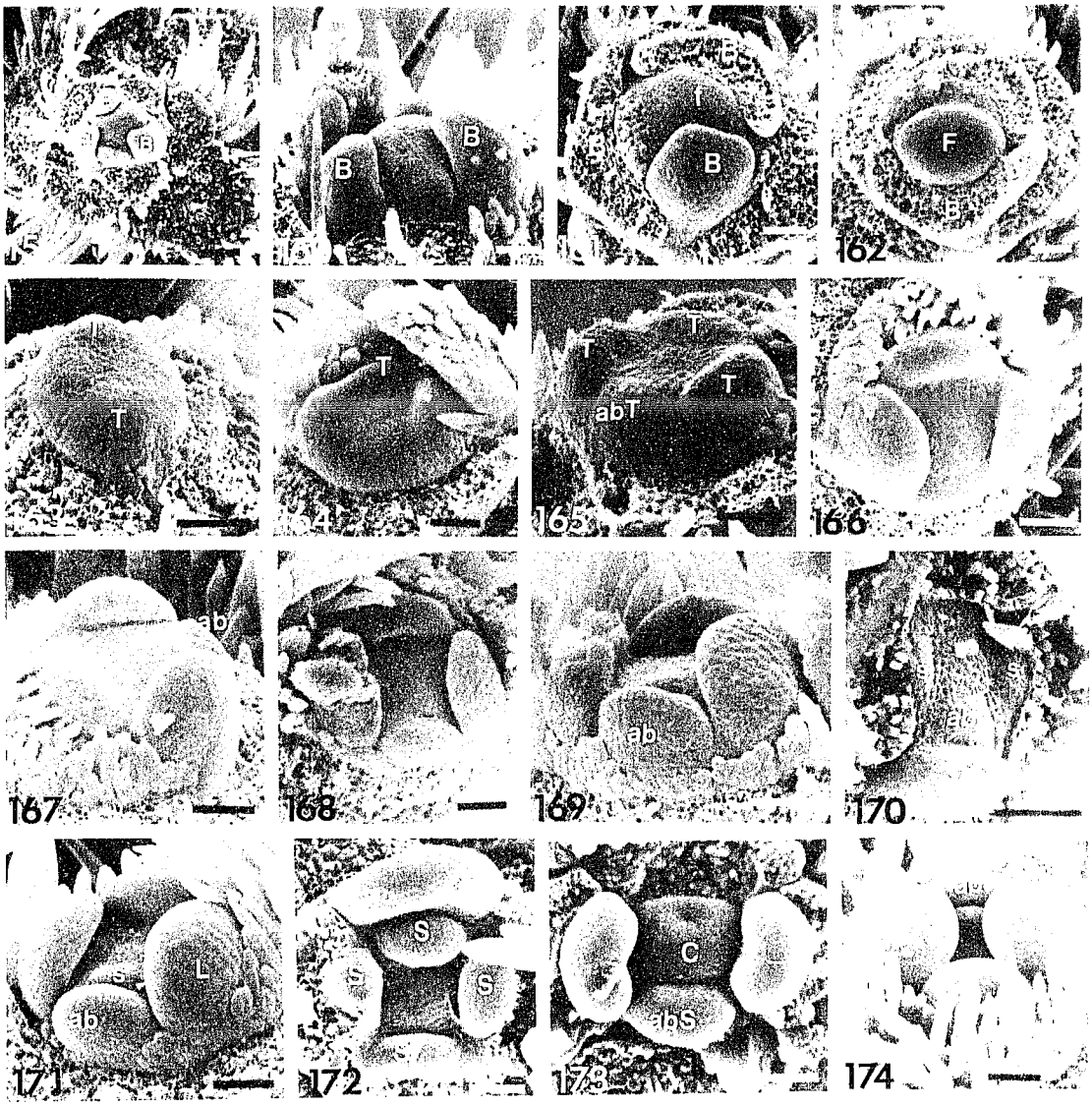
Figs. 3.157-158. Camera lucida illustrations. **157.** Mature flower and position of flower in relation to shoot. **158.** Mature anther.

stigmatic area. The receptive surface of the stigma is enclosed in a longitudinal groove that extends across the top of the carpel. The ovary is sessile and hirsute. Four nectariferous scales are present in alternitepalous positions.

Organogenesis - At the ends of vegetative shoots, five to seven (sometimes eight) inflorescence bract primordia are initiated in a phyllotactic spiral (2/5; Fig. 3.159) similar to that of the leaves. The lower bracts are lobed and leaf-like, although reduced in size, while the upper bracts are cuneate (Fig. 3.160). Within the axils of all bracts but one, a small meristem is initiated and then suppressed from enlargement. These axillary buds differentiate as trichomes (arrow in Fig. 3.161). In the axil of the second to last bract initiated, the meristem will enlarge, becoming the floral meristem (Figs. 3.161, 162). The floral meristem is laterally oblate, enlarging to approximately 140 x 100 μm before the first tepals are initiated. The first tepals to be initiated are in lateral positions, and one appears slightly larger than the other (Fig. 3.163). The third tepal is initiated in the adaxial position (Fig. 3.164) followed by the initiation of the tepal in the abaxial position (Fig. 3.165). The remaining floral meristem enlarges and increases in height on the abaxial side (Figs. 3.166, 167). A stamen is initiated in the **abaxial** position as the tepals enlarge slightly (Figs. 3.168, 169). The stamen primordia in lateral positions are initiated next (Figs. 3.170, 171) followed by the initiation of the adaxial stamen. Thus, the stamens appear to be initiated unidirectionally. A similar pattern of tepal and stamen organogenesis was observed in A. sericeus. The remaining floral meristem enlarges (Fig. 3.172), becoming approximately 90 x 75 μm during the transformation into a carpel primordium. The cleft forms adaxially on the carpel primordium (Fig. 3.173).

Organ morphogenesis - *Tepals* - Trichomes develop on the abaxial sides of the tepals (Figs. 3.171, 174). The trichomes become elongate abaxially on each tepal

Figs. 3.159-174. Inflorescence and floral organogenesis of Adenanthos oreophilus. **159, 160.** Polar and side views of inflorescence apex with bract primordia (B). **161.** Polar view of inflorescence apex. Only hairs develop in bract axils except in the second to last bract initiated in which a floral meristem (f) is initiated. **162.** Polar view of floral meristem enlarging in bract axil. **163.** Side view of floral primordium, the two lateral tepals present. **164.** Abaxial oblique view of floral primordium; the adaxial tepal has been initiated (T). **165.** Oblique abaxial view of floral primordium with four tepals. **166.** Oblique polar view of young flower showing the arrangement of the four tepals. **167.** Adaxial-side view of same showing the enlargement of the floral meristem in the abaxial half (ab). **168, 169.** Polar view and abaxial view showing the growth on the abaxial half of the flower meristem. **170.** Oblique side view showing three stamens. **171.** Oblique abaxial view showing trichomes developing on lateral tepal (L). **172.** Polar view of young flower with all four stamen primordia (s). **173.** Polar view of carpel initiation (C). **174.** Adaxial view of flower prior to aestivation. **Scale bars:** 159 = 100μm; 160-175 = 50μm.



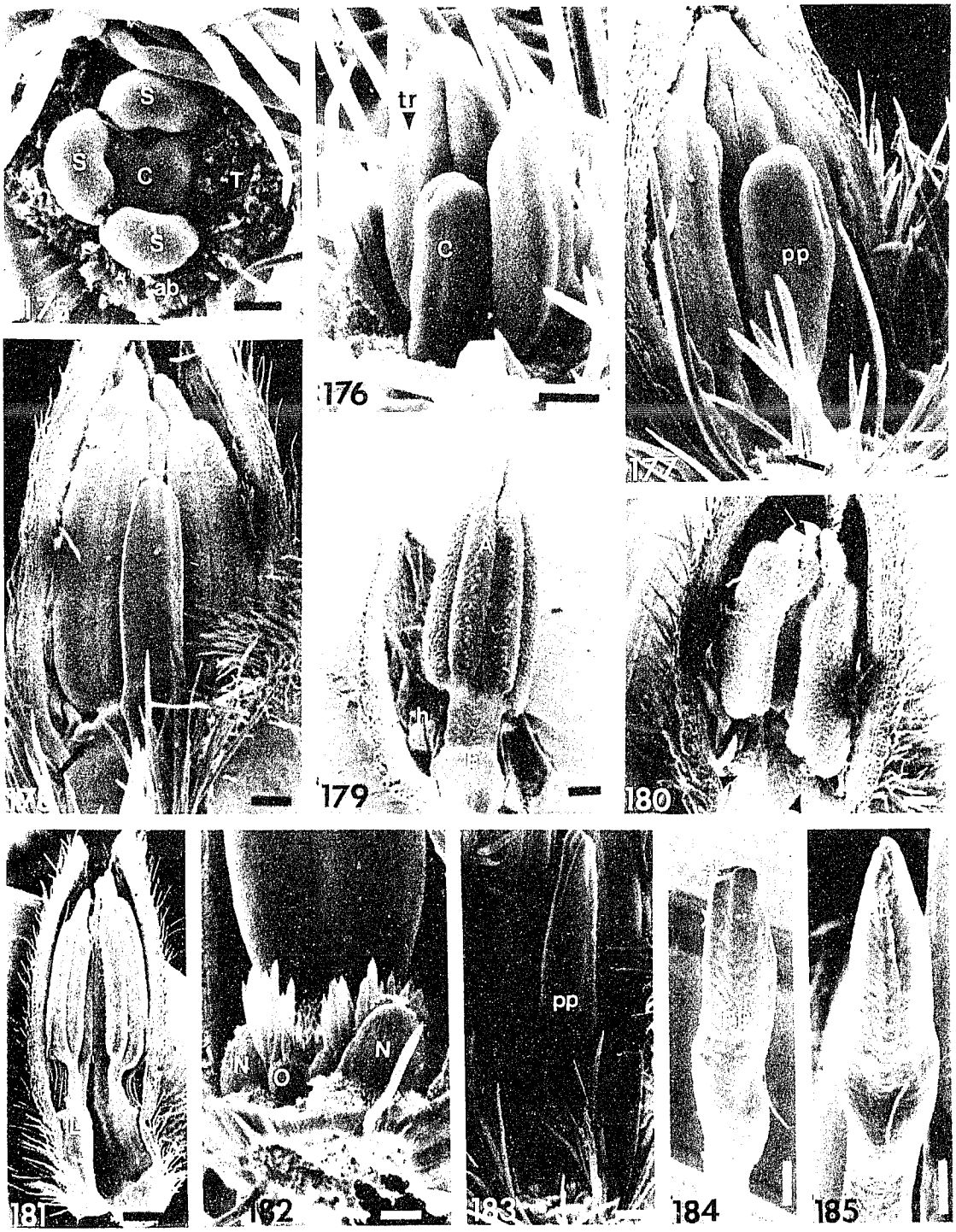
Figs. 3.159-3.174

lobe and persist through anthesis (Fig. 3.180, 181). The lateral tepals will converge and come into contact with one another over the top of the flower. The sagittal tepals converge in the corners between the points of contact of the lateral tepals and will not touch one another.

Stamens -- The median furrow develops on the lateral stamen primordia (Fig. 3.174) at a stamen height of approximately 100 μm. The median furrow forms on the adaxial and abaxial stamen primordia (Fig. 3.175). The abaxial stamen is slightly narrower than the lateral and adaxial stamen primordia (Fig. 3.175). Each stamen elongates and the transverse furrow is prominent at a stamen height of 400 μm (Fig. 3.176). A connective appendage terminates the maturing thecae (arrows in Fig. 3.176) that will be acutely tapered before anthesis (Fig. 3.180). The median furrow extends to the top of the connective appendage (arrow in Fig. 3.180). Zonal growth between and beneath the filament and tepal is present at a anther height of one mm (Figs. 3.177, 178). Lobes develop on the basal and lateral portions of each filament at the tepal/filament interface (arrow in Fig. 3.178). Elongate trichomes differentiate behind the short filaments and on the adaxial surface of the tepals (Fig. 3.179). The epidermis of the microsporangia becomes verrucate or papillate (Fig. 3.179). The filaments are short. The anthers are introrse and have longitudinal dehiscence lines (Fig. 3.158).

Carpel -- The carpel enlarges (Fig. 3.175) and increases in height. The margins of the carpel enlarge and the cleft extends across the summit of the carpel primordium (Fig. 3.176). The upper portion of the young carpel enlarges marking the early stages of form differentiation of the pollen-presenter (Fig. 3.177). Trichomes develop on the top of the enlarged sessile ovary (arrow in Fig. 3.177). At a later stage, the ovary has enlarged and an additional hair type, that is narrower and shorter than the ovarian hairs, begins to develop on the proximal portion of the style (Fig. 3.182). The cleft does not extend to the carpel base. The upper portion of the carpel continues to enlarge and

Figs. 3.175-185. Floral morphogenesis of Adenanthos oreophilus. **175.** Polar view of floral organ arrangement (T=tepals; S=stamens; C=carpel; ab=abaxial side). **176.** Side view of maturing stamens (tr=transverse furrow) and enlarging carpel. **177.** Side view of older flower showing first signs of pollen-presenter (pp) development. Hairs are differentiating on ovary (arrow) and there is an appendicular connective (ap) atop the stamens. **178.** Side view of upper portion of flower showing pollen-presenter enlargement. **179.** Upper portion of tepal and stamen. The anther (A) surface is verrucate. The filament (F) is lobed and elongate hairs (h) develop behind the filament. **180.** Oblique polar view of anthers in bud. Arrowheads point to filament lobes that are fused to neighboring filaments. **181.** Side view of tepals and anthers showing the lobed filaments (L). **182.** Base of ovary (O) showing the two types of hairs and the presence of nectary lobes (N). **183.** Side view of enlarging pollen-presenter (pp). **184.** Side view of pollen-presenter (pp) showing the mature stigma (st). **185.** Adaxial view of pollen-presenter and the closed stigma (st). **Scale bars**: 175 = 50μm; 176, 177, 182 = 100μm; 178-180, 183-185 = 200; 181 = 500μm.



Figs. 3.175-3.185

elongate (Fig. 3.178). The style is narrow and constricted just above the ovary and becomes laterally broad higher up, eventually tapering towards the developing and broadening pollen-presenter (Fig. 3.183). The young pollen-presenter continues to elongate and broaden (Fig. 3.178, 183). The base of the pollen-presenter becomes slightly lobate at about the same level as the young filaments of the anther (arrows in Fig 183). Distal to the lobes, the pollen-presenter is laterally flattened or sagittally broad. The shape of the pollen presenter persists through anthesis (Fig. 3.184, 185). The stigma is a groove that extends across the top of the carpel and down the ventral side for a short distance (arrow in Fig. 3.184). When receptive, the stigmatic margins will separate slightly (Nelson, 1978). Four cuneate nectariferous scales develop (Fig. 3.182) from the floral base in alternitepalous positions when the flower is approximately one mm high. The lobes become elongate and thin at anthesis.

Sect. Eurylaema - Adenanthos obovata (Figs. 3.186-237) - Organography - A. obovata is found in Western Australia and is a scrubby shrub with a lignotuber (Nelson, 1978). The shoots appear monopodial and auxotelic. Two buds form within the axil of each leaf and are positioned vertically. The bud that is proximal to the leaf is a dormant vegetative bud that will develop if the first-order shoot is clipped (pers. obs.) or destroyed by a fire (Nelson, 1978; Wrigley and Fagg, 1988). The second meristem, distal to the dormant vegetative bud, is a reproductive or inflorescence shoot and differs in position and growth. Two sclerified prophylls and five, or more regularly, six sub-opposite decussate bracts are on each unit inflorescence. The pedunculate internode between the prophylls and the first two bracts is elongate.

A single, sessile, flower develops within one of the bract axils. The single red-orange flower on each inflorescence axis is highly zygomorphic (Fig. 3.186). The perianth arches towards the subtending leaf. The style similarly arches towards the leaf.

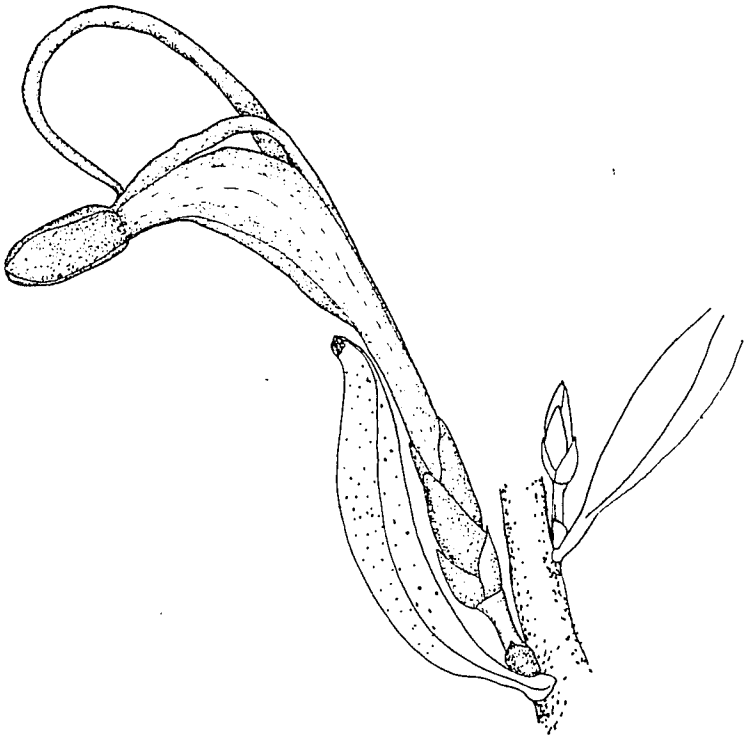
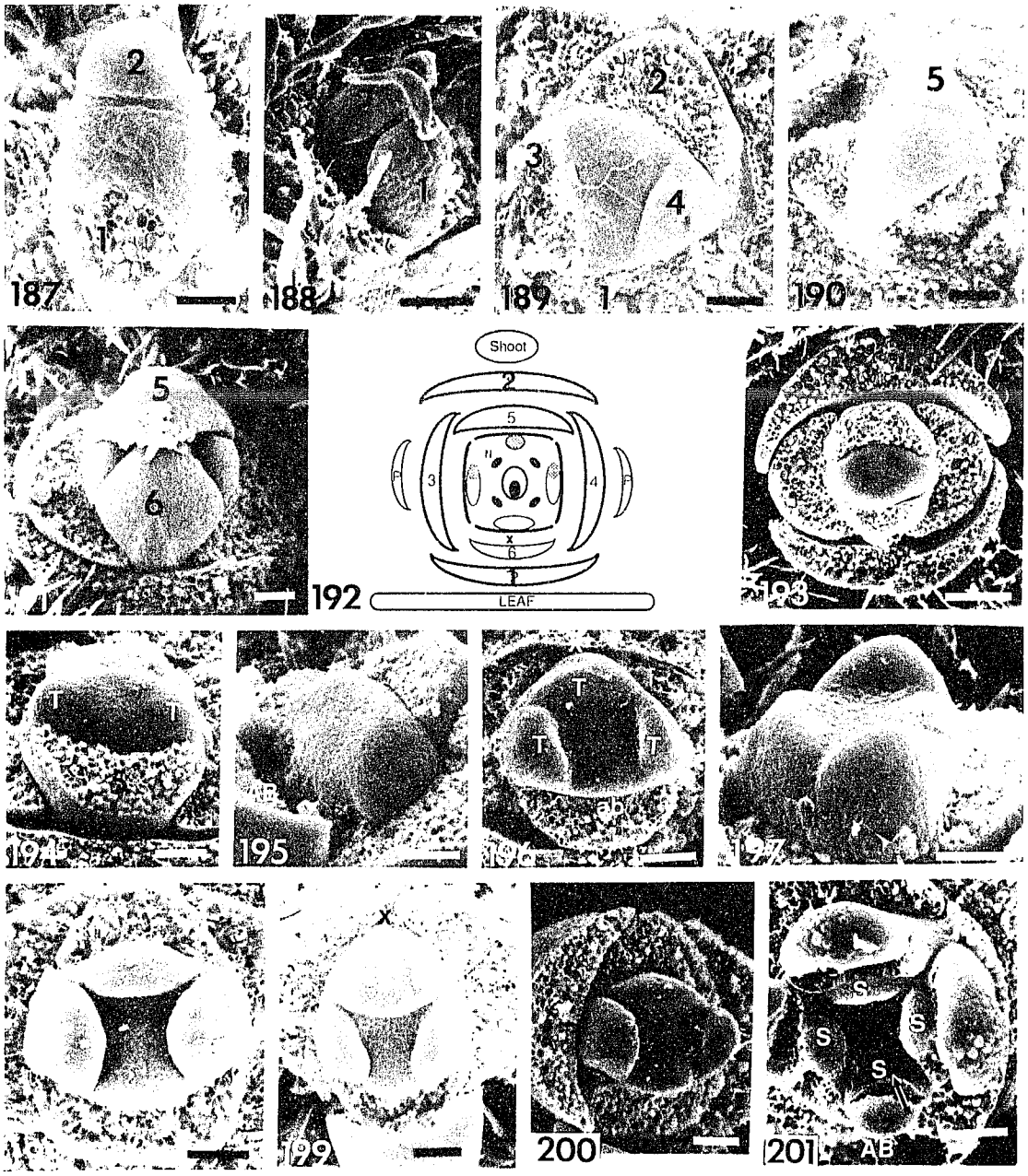


Fig. 3.186. Camera lucida illustration of flower and inflorescence arrangement on shoot in Adenanthos obovatus.

The pollen presenter is spatulate, being laterally wide and sagittally flat. The adaxial anther is fertile as are the upper portions of the adaxial halves of each lateral anther. The abaxial halves of each lateral anther are sterile (staminodia) as is the abaxial anther. It should be noted that the perianth and carpel arch **adaxially** in relation to the subtending floral bract, not the subtending leaf.

Organogenesis - From the lateral flanks of the inflorescence meristem, two prophylls are initiated followed by the initiation of five to six bracts in a sub-opposite decussate pattern. The first two bracts are initiated in the sagittal plane, one adaxially positioned and the other abaxially positioned in relation to the leaf (Figs. 3.187, 188). The third and fourth bracts are initiated next, one on each side (Fig. 3.189), followed by the initiation of the fifth bract in an adaxial position (Fig. 3.190). On some inflorescences, a sixth bract is initiated in an abaxial position on the inflorescence (Fig. 3.190). The arrangement of the bracts and flower topology is diagrammed in Figure 192. In the axil of the fifth bract, the meristem will broaden and become a floral primordium (Fig. 3.192). Within the axils of the other bracts, a group of trichomes differentiate (arrows in Figs. 3.208, 217). The floral meristem enlarges (Fig. 3.193) becoming approximately 160 x 100 μm before the initiation of the tepals. The first two tepals are initiated in lateral positions and appear simultaneously (Fig. 3.194, 195). The third tepal is initiated in the adaxial position (Fig. 3.196). The tepal in the abaxial position is initiated last (Figs. 3.197-200). The width of the adaxial tepal varies on different inflorescences; in five-bracted inflorescences, the adaxial tepal is generally thicker (Figs. 3.197, 199) than the adaxial tepal on six-bracted inflorescences (Figs. 3.198, 200). The apparent difference in relative width is probably a result of available space on the inflorescence meristem. The floral meristem, after tepal initiation, is higher abaxially (arrow Fig. 3.200). Order of initiation of all stamens could not be identified. The stamen in the abaxial position however appears to be initiated last

Figs. 3.187-201. Inflorescence and floral organogenesis in Adenanthos obovatus. **187.** Polar view of early inflorescence apex after the first two bracts have been initiated (1,2). **188.** side view of first two bracts over inflorescence apex. **189.** Polar view of inflorescence apex with the first two bracts removed and the third and fourth present. **190.** Polar view of inflorescence apex, the fifth bract initiated in an adaxial position relative to the leaf (which is at bottom in the image). **191.** Polar view of inflorescence. The apex is covered by the fifth and sixth bracts. **192.** Drawing of tetrastichous arrangement of bracts on inflorescence in relation to flower. **193.** Polar view of floral meristem. **194.** Oblique abaxial view of same showing the first two tepals (T) in lateral positions. **195.** Oblique side view of floral primordium with two tepals. AB=abaxial side of flower. **196.** Polar view of third tepal being initiated in a five-bracted inflorescence (x = missing bract on adaxial side of inflorescence). **197.** Side view of flower showing the fourth tepal being initiated in the abaxial position (abT). **198.** Polar view of flower on a six-bracted inflorescence. **199.** Adaxially oblique view of flower in a five-bracted inflorescence. **200.** Oblique side view of four-tepallate flower on a six-bracted inflorescence. **201.** Polar view of flower after stamen (s) initiation. **Scale bars:** 187 = 25μm; 188-201 = 50μm.



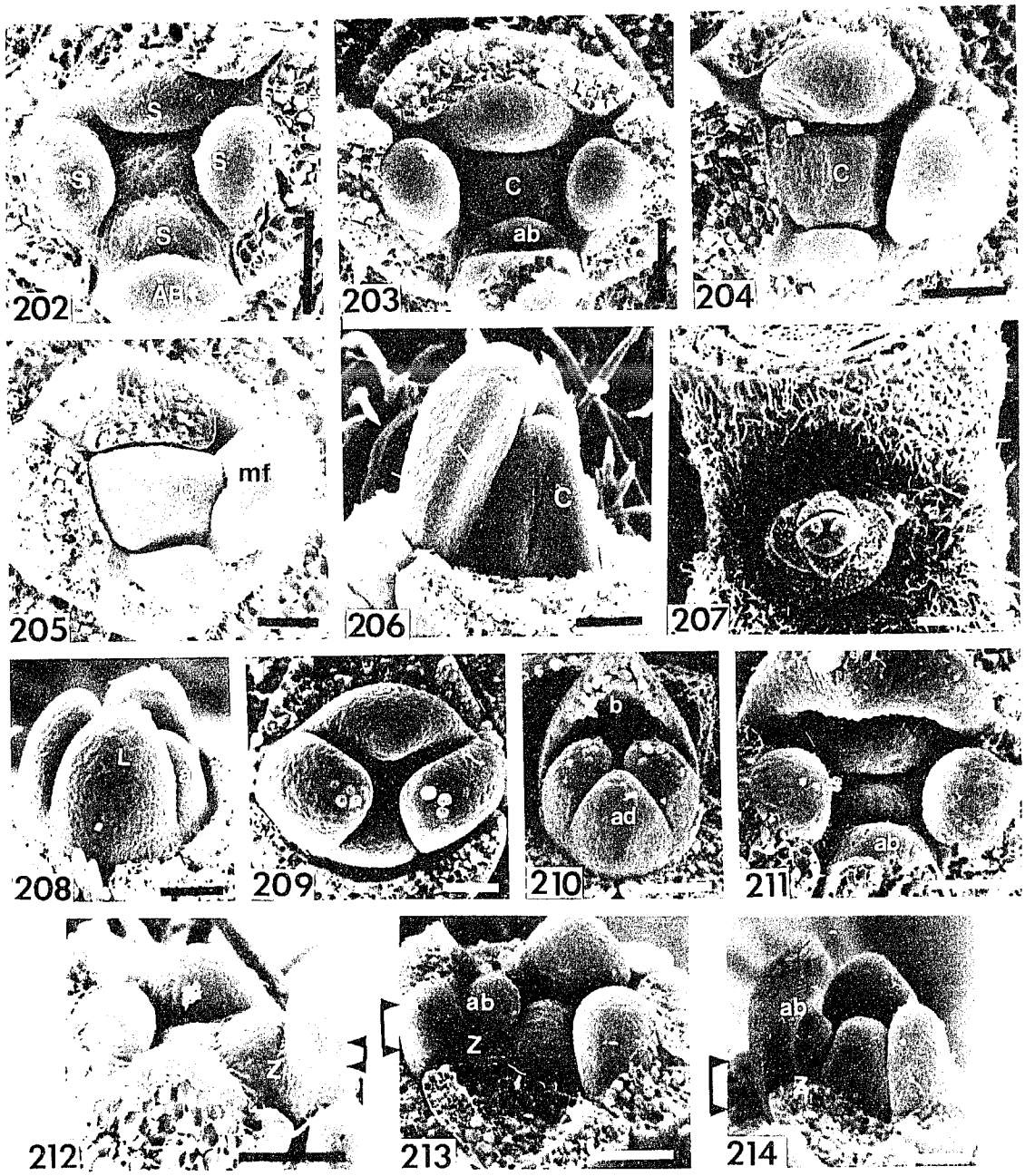
Figs. 3.187-3.201

(arrow, Fig. 3.201). The remaining floral meristem is laterally broad (Fig. 3.202; 40 x 30 μm) and enlarges to approximately 75 x 50 μm during the transition to a carpel primordium (Figs. 3.203, 204). The cleft forms on the adaxial side (Figs. 3.205, 206) when the carpel primordium is approximately 100 μm high. In relation to the leaf, the flower is upside down (Fig. 3.192, 207).

Organ morphogenesis - *Tepals* - Trichomes develop on the upper part of the abaxial side of each tepal. Among tepals, they develop in a similar sequence as that in which the tepals were initiated. Trichomes first develop on the lateral tepals (Fig. 3.208, 209) followed by the differentiation of trichomes on the adaxial tepal (Fig. 3.210) and lastly on the abaxial tepal (Fig. 3.214). The lateral tepals will converge (Fig. 3.209, 210) and come into contact with one another over the top of the flower. The sagittal tepals converge in the corners between the points of contact of the lateral tepals and do not touch one another. The trichomes elongate and continue to develop abaxially (Fig. 3.229).

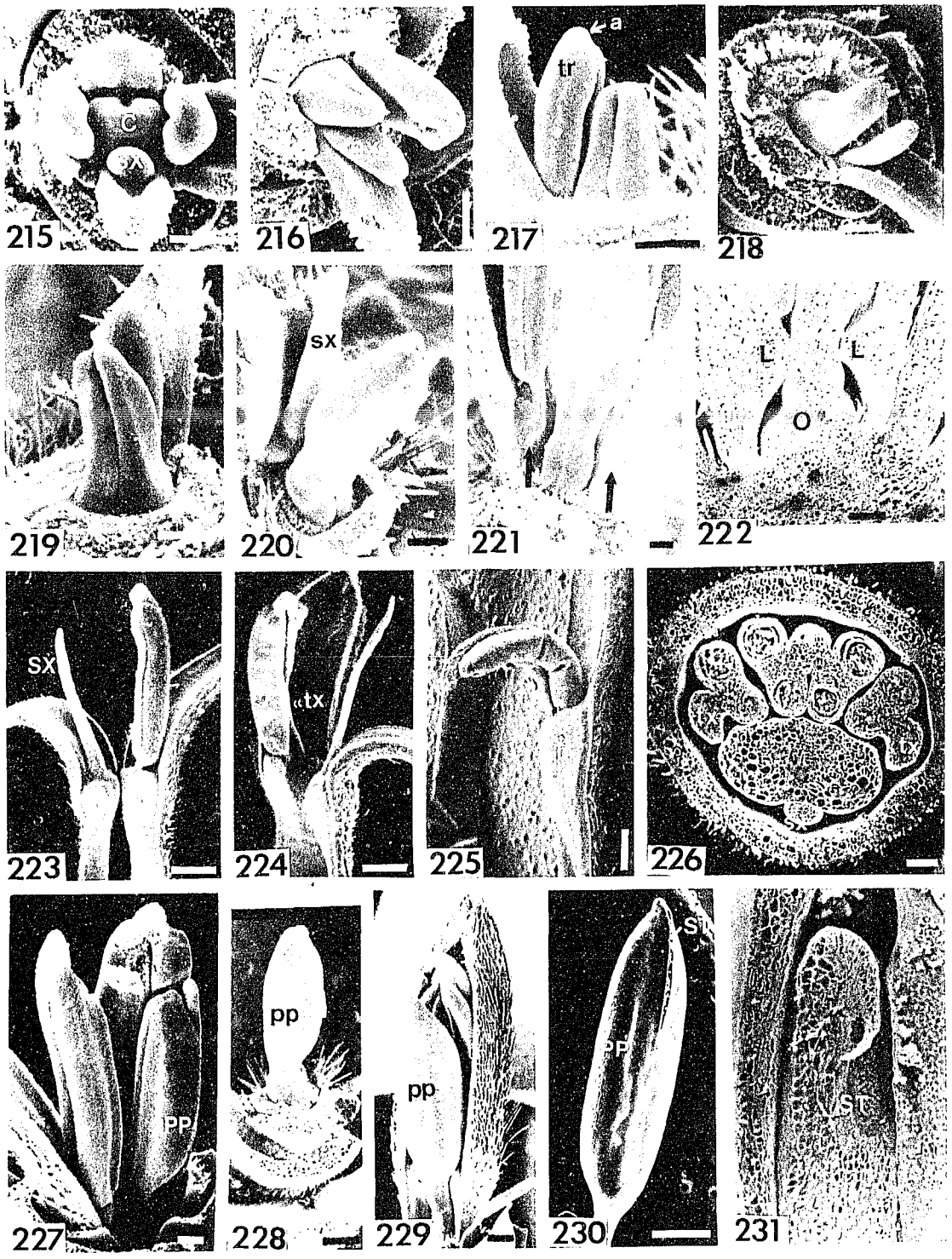
Stamens - The stamen primordia are not equal in size after initiation. The abaxial primordium is the smallest (Figs. 3.202-204). Zonal growth beneath and between the abaxial tepal and abaxial stamen primordium (bracketed arrows in Figs. 3.212-214) occurs before the same process in the other stamens and tepals. The precocious growth on the abaxial side results in an obvious dorsiventral change in symmetry of the young flower (compare bracket length in Figs. 3.212-214). The adaxial and lateral stamen primordia broaden (Figs. 3.204, 205) and the median furrow develops on the lateral stamen primordia at a stamen height of approximately 150 μm (Fig. 3.205). The median furrow forms on the adaxial primordium subsequently (Fig. 3.215). The transverse furrow develops on the lateral stamen primordia (Fig. 3.217) and adaxial stamen primordium at a stamen height of approximately 275 μm.

Figs. 3.202-214. Organogenesis and morphogenesis of flowers of Adenanthos obovatus. **202.** Polar view of flower with all four stamen primordia (S). **203.** Polar view of early carpel initiation (C). The abaxial stamen primordium (ab) is smaller than the others. **204.** Enlargement of the early carpel primordium (C). **205.** Polar view of flower showing the cleft on the adaxial side of the carpel primordium (C). The median furrow (mf) is present on the anther. **206.** Adaxial view of carpel showing the cleft. **207.** Polar view of flower on inflorescence attached to stem: the leaf is at the bottom of the picture. **208.** Side view of flower with trichomes (arrows) differentiating on lateral tepals (L). **209.** Polar view of tepals prior to aestivation. **210.** Adaxial view of flower showing subtending bract (b) and trichomes differentiating on the adaxial tepal (ad). **211.** Polar view of flower before carpel initiation, illustrating the smaller abaxial stamen primordium (ab). **212.** Side view of flower after stamen initiation showing the height difference between the abaxial and adaxial half as well as the precocious zonal growth (Z) between the abaxial stamen primordium and tepal. **213.** A later stage showing the smaller abaxial stamen primordium and the zonal growth (z). **214.** Side view of later stage showing the height difference due to zonal growth (z) between the abaxial stamen (ab) and tepal. **Scale bars:** 202-206, 208-214 = 50μm; 207 = 200μm.



Figs. 3.202-3.214

Figs. 3.215-231. Floral morphogenesis in Adenanthos obovatus. **215.** Polar view showing structure of flower. The abaxial stamen (SX) primordium is smaller and will be sterile. **216.** Oblique side view of enlarging flower. **217.** Adaxial view of carpel and anther. Hairs (h) developed in the axil of the sixth bract primordium (removed). There is an appendicular connective (a) on the top of the young anther. The transverse furrow (tr) is present. **218.** Oblique side view of carpel, abaxial staminode and abaxial tepal (lower right). Zonal growth between the tepal and staminode is lifting the staminode. **219.** Adaxial view of carpel showing enlargement. **220.** Oblique side view of early pollen-presenter formation. The abaxial staminode (SX) is elongate. **221.** Adaxial view of lower portion of flower showing the development of lobes (arrows) on the filaments at the tepal/filament insertion. **222.** Longi-section of flower showing the enlargement of the lobes (L). **223.** Preanthesis lateral anther and abaxial staminode (SX). The filament lobes (L) are large. **224.** Alternate view of flower in 223 showing the partial sterility of the abaxial side of the lateral anther (tx). **225.** Adaxial anther before dehiscence showing the filament lobes (L). **226.** Cross-section of preanthesis bud. The adaxial anther (top) is fertile; the adaxial thecae of the lateral anthers are fertile; the abaxial thecae of the lateral anthers (tx) are sterile as is the abaxial staminodium (sx). **227.** Oblique side view showing the pollen-presenter (pp). **228.** Abaxial view of carpel with a broad pollen-presenter (pp). **229.** Pollen-presenter broadening. **230.** Mature pollen-presenter is spatulate and the stigma (ST) is a slit along the adaxial side. **231.** Longitudinal section through developing stigma showing the stigmatic groove (ST). **Scale bars:** 215-222, 226, 231 = 100μm; 223-225, 230 = 500μm; 227-229 = 200μm.



Figs. 3.215-3.231

During stamen morphogenesis, the abaxial primordium has a different developmental fate than the other stamens. The abaxial stamen primordium is suppressed from becoming fertile. It enlarges and becomes laminar in shape (Fig. 3.216). The abaxial stamen elongates as zonal growth beneath the tepals lifts the anthers (Fig. 3.220) and at maturity will be approximately the same length as the lateral anther (Fig. 3.223, 224).

The lateral stamens and the adaxial stamen have similar developmental pathways until an anther height of approximately two mm. A connective appendage elongates atop the maturing anthers (arrow in Fig. 3.217). The connective appendage becomes rounded prior to anthesis (Figs. 3.227, 224, 225). Zonal growth between and beneath the filaments and tepals occurs at a stamen height of 1.5 mm (already present in Fig. 3.222). Lobes develop on the basal and lateral portions of each filament at the tepal/filament interface (arrow in Fig. 3.221). The lobes broaden (Fig. 3.222) and each side of the lobe comes into contact with the lobes of the neighbouring tepal/filament (arrows, Fig. 3.223). The epidermis of the lobes is modified so that the cells interlock with those of the neighboring lobes (Fig. 3.223). There is little elongation of the filaments subtending each of the lateral and adaxial anthers (Fig. 3.223-225). At a height of approximately 2 mm, after the differentiation of epidermal verrucae, the abaxial thecae of the lateral anthers are narrow in relation to the adaxial thecae (Fig. 3.224, 226). In most flowers, no pollen is produced in the abaxial microsporangia of each lateral anther (Fig. 3.226). In some flowers, pollen is produced but is limited to the upper part of each abaxial theca (Venkata Rao, 1960). The microsporangia of the adaxial anther (Fig. 3.190) produce pollen. The anthers are introrse and have longitudinal dehiscence lines.

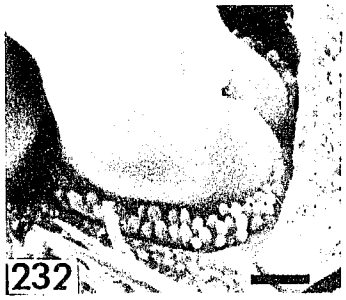
Carpel -- The carpel enlarges and increases in height (Fig. 3.216). The cleft does not extend quite to the carpel base (Fig. 3.219). The cleft does not extend across the top

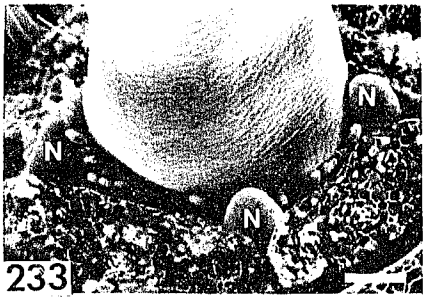
of the carpel (Fig. 3.216). The margins of the carpel enlarge (Fig. 3.219) and at a carpel height of approximately 300 μm the cleft extends to the top of the carpel primordium (Fig. 3.220). Two lobes form from the cleft margins at the base of the carpel (arrows in Fig. 3.218, 219) at a carpel height of approximately 300 μm. The base of the carpel continues to enlarge forming the ovary (Fig. 3.220). The lateral sides of the upper portion of the carpel enlarge that will become the pollen-presenter at a similar stage (Fig. 3.220). The lateral sides continue to enlarge through development (Figs. 3.227-229) and at maturity, the pollen presenter is sagittally flat and longitudinally spatulate (Fig. 3.230). Stigmatic papillae form in the upper portion of the carpel cleft (Fig. 3.231) and are enclosed by the margin until receptivity at which time, the margins separate (Fig. 3.230; Nelson, 1978). Trichomes develop from the floral receptacle around the enlarging ovary (Figs. 3.220, 232). Above the broadening ovary, and beneath the developing pollen-presenter, the style elongates (Fig. 3.221, 228, 229). Trichomes develop on the base of the style above the ovary at a similar time that trichomes develop in acropetal order from the base of the ovary (Fig. 3.234). At maturity, the trichomes are elongate and thin (Fig. 3.236).

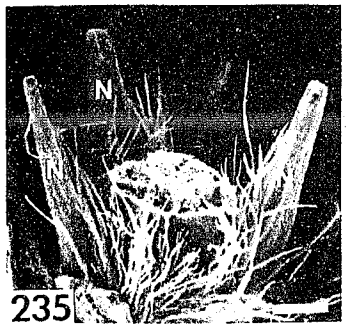
The floral receptacle enlarges and heightens around the carpel base (Fig. 3.232). Four nectariferous scales develop in alternitepalous positions from the broadened receptacle when the flower is approximately 800 μm high (Fig. 3.233). The lobes enlarge after initiation (Fig. 3.234) becoming elongate (Fig. 3.235) and tapered at anthesis (arrow in Fig. 3.236).

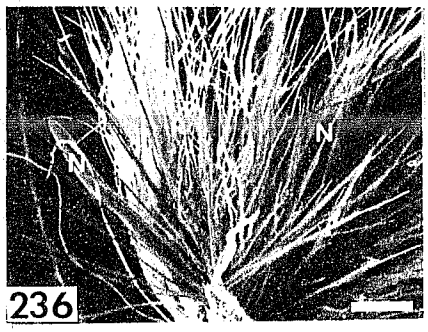
Proteae - Proteinae - There are 11 genera in the African Proteinae (Rourke, 1980, 1984, 1988). There are two genera in the other subtribe (Aulaciinae; Johnson and Briggs, 1975). The flower morphology of Faurea speciosa, Mimetes floribundus, Orothamnus zeylandica, Protea nerifolia Leucospermum reflexum, Vexatorella obtusata

Figs. 3.232-236. Nectary development in Adenanthos obovatus. **232.** Base of ovary and receptacle. Hairs developing. **233.** Nectaries (N) are initiated from a short hypanthium in alternitepaolous sites. **234.** Enlargement of the nectaries and the elongation of ovary and stylar hairs. **235.** Nectaries before anthesis. **236.** Nectaries at anthesis (N). **Scale bars:** 232, 233 = 50μm; 235, 236 = 100μm; 236= 500μm..









Figs. 3.232-3.234

has been examined and compared from mature fixed material. The ontogeny of Serruria pedunculata is presented.

Serruria pedunculata - (Figs. 3.238 -273) - Organography -- There are over 46 species in the genus (Rourke, 1984a, b; Wrigley and Fagg, 1988). Plants of Serruria are sclerophytic shrubs with highly dissected, usually terete, leaves. In Serruria pedunculata, the blastotelic inflorescences are terminal on the shoot. Dormant vegetative buds are initiated in the axils of the leaves (usually reduced) subtending the inflorescence and will develop after flowering. Over 100 flowers develop on a capitate inflorescence. The lower bracts of the inflorescence in some other species can be elongate and colorful; a showy involucre.

The tepals of the sessile flowers are are approximately equal in length and densely hirsute. The flowers are elongate. The upper half of the four tepal lobes reflex away from the longitudinal axis of the flower at anthesis. The stamens are adnate to the perianth lobes. Each stamen has a very short filament and basifixed tetrasporangiate anthers. The anther is introrse and dehisces via longitudinal lines. There does not appear to be a sterile connective appendage distal to the microsporangia. The abaxial microsporangia are longer than the adaxial microsporangia of each anther. The carpel is elongate at anthesis. The pollen-presenter is tapered and swollen, almost club-like. The stigma is represented by an adaxial groove that extends across the top of the carpel. The style is elongate. The ovary is sessile and hairy. There are four nectariferous glands present in alternitepalous positions. Partial developmental series were obtained for S. floridana; the floral ontogenies were very similar to those of S. pedunculata.

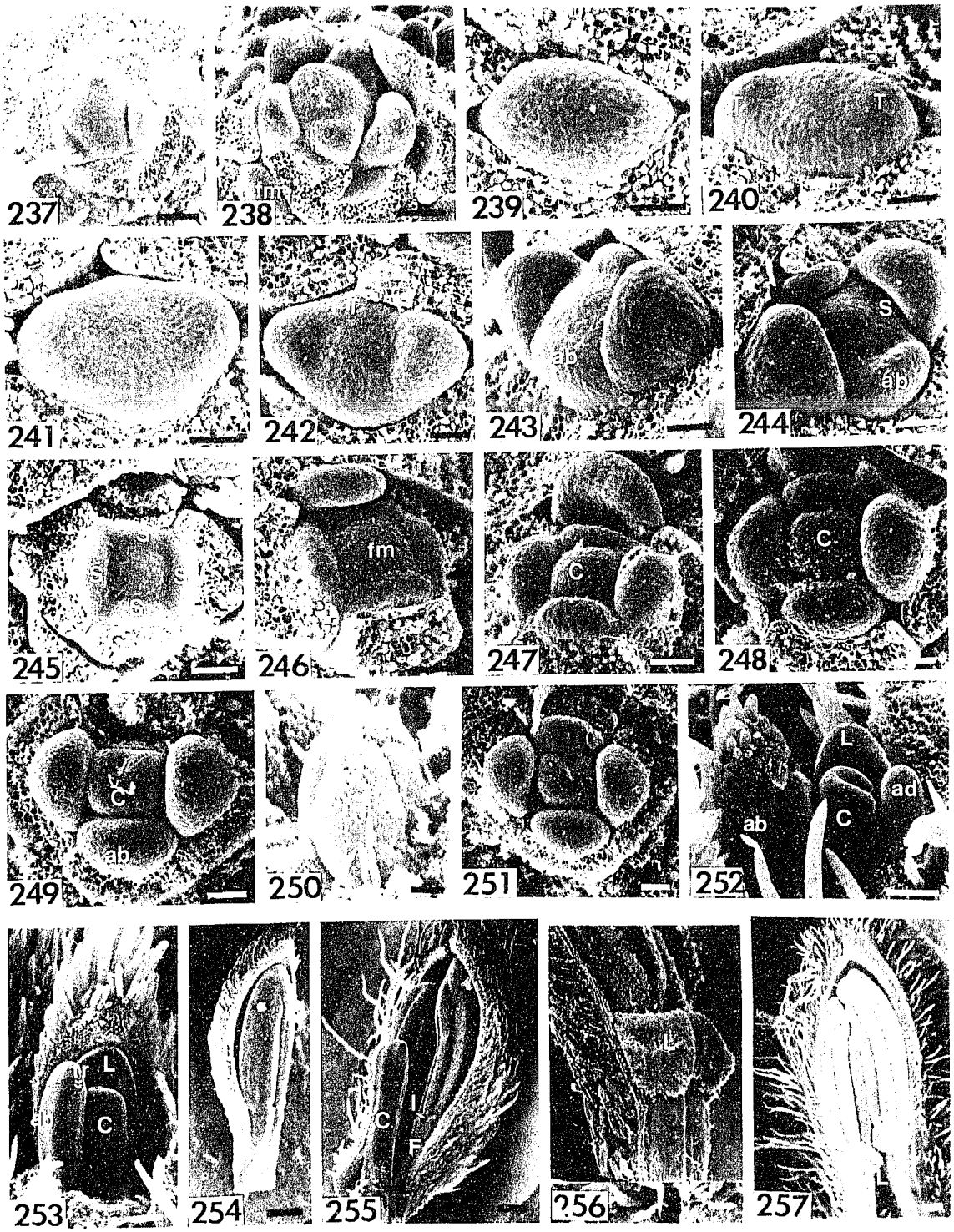
Organogenesis - The inflorescence meristem initiates bract primordia in a 3/8 anthotactic spiral (Fig. 3.237). Within the axil of each bract, a floral meristem is initiated (Fig. 3.238), becoming laterally oblate and approximately 175 x 110 μm (Fig.

3.239) prior to organogenesis. The lateral tepals appear to be initiated sequentially although rapidly in relation to one another (Figs. 3.240, 241). The sagittal tepals also appear to be initiated sequentially, the tepal in the adaxial position (Fig. 3.242) initiated prior to the tepal in the abaxial position (Fig. 3.243). The stamens are initiated in a similar sequence as the tepals in epitepalous positions. The lateral stamens (Fig. 3.244) precede the sagittal stamens (Figs. 3.245, 246). The abaxial stamen appears prior to the adaxial stamen in most flowers (Figs. 3.245, 246). The remaining floral meristem (Fig. 3.247) has a width to breadth ratio of 1.06 and enlarges to a width of approximately 95 μm and a breadth (sagittally) of 90 μm (Fig. 3.248) prior to the inception of the carpel . The cleft forms adaxially on the carpel primordium (Fig. 3.249).

Organ morphogenesis -*Tepals* - The lateral tepals converge above the remaining floral organs and come into contact with one another. The sagittal tepals similarly arch over the remaining floral organs but the distal tips do not come into contact with one another (Fig. 3.250). Trichomes develop on the top portion of the tepals in the same sequence they were initiated (Fig. 3.250). As the tepals elongate, trichomes differentiate basipetally on the abaxial surface of the tepal (arrows Fig. 3.255). The trichomes become elongate through anthesis (Fig. 3.253, 255).

Stamens - The lateral stamen primordia become larger than the sagittal stamen primordia in width, breadth (Fig. 3.251) and height (Fig. 3.252). The stamen primordia enlarge to a height of approximately 200 μm before the median furrow demarcates the thecae. The transverse furrow is present when the stamens are 400 μm high (Fig. 3.253). As the stamens develop, the transverse furrow extends to the top of the primordia (Figs. 3.254, 255). No connective appendage develops. The filament becomes evident (Fig. 3.255), as does the zonal growth beneath and between each tepal and stamen (arrow in Fig. 3.254). As zonal growth continues, resulting in the lifting of

Figs. 3.237-257. Floral organogenesis and morphogenesis in Serruria pedunculata. **237.** Polar view of inflorescence apex initiating bracts . **238.** Oblique view of inflorescence apex showing floral meristems (fm) developing in bract axils. **239.** Floral apex in bract axil. **240.** Initiation of the first two tepals (T) in lateral positions. **241.** Two tepals present in lateral positions. **242.** The tepal (T) in the adaxial half of the flower is initiated. **243.** Oblique view of floral primordium: the abaxial tepal is present (ab). **244.** Oblique view showing the lateral stamen (S) being initiated. **245.** Polar view after all four stamens (S) are initiated. **246.** The remaining floral meristem (fm) enlarges. **247.** Oblique view showing early carpel (C) development. **248.** Enlargement of the early carpel. **249.** The carpel cleft forms on the adaxial side (opposite 'ab'). **250.** Side view of bud at aestivation. **251.** Polar view of enlarging flower. **252.** Side view of flower showing height of carpel. ab=abaxial side; L=lateral stamen; ad=adaxial stamen. **253.** Side view of flower showing height of carpel (C) and abaxial (ab) and lateral (L) stamens. The transverse furrow (tr) is present. **254.** Removed anther and tepal showing zonal growth (z) region and lobes on the filament. **255.** Partially dissected bud showing carpel (C) and stamens. The filament (F) is lobed (l). **256.** Mature anther showing the lobed filament. **257.** Two anthers separated from flower showing fusion at lobed filaments (L). **Scale bars:** 237-239, 252, 253 = 100; 240-251 = 50μm; 254-257 = 200μm.



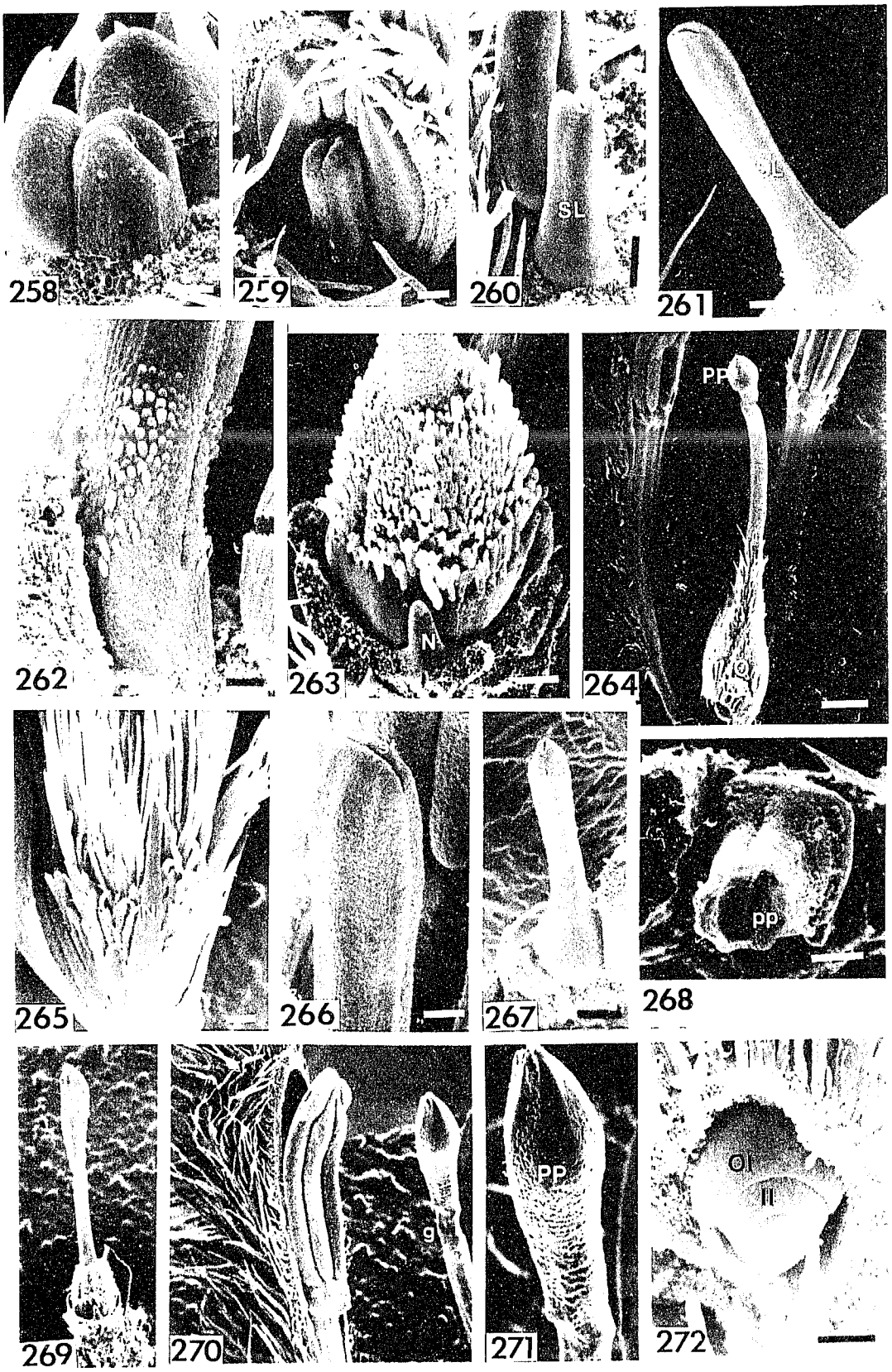
Figs. 3.237-3.257

the stamen relative to the floral base, the top of the short filament becomes lobed (Fig. 3.254). Two lobes develop on the adaxial side of the filament and one lobe develops on each side of the filament just above the point of tepal/stamen adnation (Fig. 3.255). The lobes enlarge (Fig. 3.256) and fuse to the neighbouring tepal/filament lobes at different heights (arrows in Fig. 3.257). The epidermis of the anthers becomes slightly verrucate. Prior to anthesis, the anthers dehisce along longitudinal lines (basal portion of anther in Fig. 3.256), depositing their pollen on the pollen presenter (Fig. 3.269).

Carpel -- The carpel cleft does not extend to the carpel base (Fig. 3.258). The carpel enlarges and increases in height (Fig. 3.252). The cleft extends across the top of the carpel through continued enlargement of the top portion of the carpel (Figs. 3.253, 259, 260). The base of the carpel starts to broaden at a carpel height of 500 μm (Fig. 3.259). The carpel continues to elongate, and hairs develop above the carpel base at a carpel height of 1300 μm (Fig. 3.261). Hairs continue to differentiate both acropetally up the enlarging style and basipetally down the enlarging ovary (Figs. 3.262, 263). At maturity, the ovary and basal portion of the style are cloaked in elongate hairs (Figs. 3.264, 265).

The top portion of the carpel appears to broaden at a carpel height of 500 μm (Fig. 3.261). The top portion of the carpel represents the early stages of the pollen-presenter. It continues to broaden. First in the sagittal plane (Fig. 3.266) and then in the frontal plane (Figs. 3.267, 268). The pollen presenter appears as an elongate swollen club at a carpel height of 3 mm. (Fig. 3.269). The style continues to elongate and the pollen-presenter fits in the concavity formed by the anthers (Fig. 3.257). Similarly, the distal portion of the style, proximal to the pollen-presenter, is clasped by the swollen lobes on the filaments (Fig. 3.270). At maturity, the pollen-presenter is club-like and the stigmatic region is on the distal and adaxial terminus of the carpel (Fig. 3.272). The

Figs. 3.258-272. Floral morphogenesis of Serruria pedunculata. **258.** Side view of carpel. The cleft does not extend down the adaxial length of the carpel. **259.** Adaxial oblique view of carpel. **260.** Abaxial view of elongating style (sl). **261.** Elongating carpel showing hairs on ovary (O). **262.** Lower portion of carpel showing hairs differentiating on the ovary. **263.** Nectaries (N) initiated in alternitepalous positions on a short hypanthium around the ovary. **264.** Flower cut open showing hairy ovary and pollen-presenter. **265.** Mature nectaries (N). **266.** Enlargement of the pollen-presenter and stigma (st). **267.** Side view of carpel showing the enlargement of the pollen-presenter (pp). **268.** Polar view of pollen-presenter (pp). **269.** Enlargement of the pollen-presenter. **270.** Mature anther and pollen-presenter: the base of the pollen-presenter is grooved (g). **271.** Mature pollen-presenter. **272.** Ovule. **Scale bars:** 258, 262 = 50μm; 259-261, 263, 265-268, 271, 272 = 100μm; 264, 269 = 500μm; 270 = 200μm.



Figs. 3.258-3.272

stigmatic surface is enclosed by the carpel margins. At anthesis, the pollen-presenter holds the pollen. The stigmatic margins will separate slightly when they become receptive (Venkata Rao, 1960). There is one slightly hemitropous (more probably orthotropous) bitegmic ovule in the loculus (Fig. 3.272; Venkata Rao, 1960).

Four nectary primordia are initiated in alternitepalous positions on a concave floral receptacle or short hypanthium (Fig. 3.263) well after organogenesis. The nectariferous lobes elongate and become acutely tapered and thin at anthesis (Fig. 3.265).

DISCUSSION

The mature flowers of Proteoideae are morphologically diverse. The flowers can have relatively simple and straightforward organizations of parts as found in (Symphionema, Isopogon, and Serruria), or they can have diverse and complex organizations. Ontogenetic analyses provide a simple and relatively easy method to elucidate complex floral morphologies, because early developmental stages of flowers are generally easier to interpret than mature forms. Knowledge of the developmental processes of flowers provides a means to examine and compare putatively homologous structures among flowers. Ontogenetic comparisons are useful in providing an empirically derived framework that can be used to evaluate convergences and divergences of characters among taxa, based on specific differences and similarities in the temporal and spatial ontogenetic patterns. The developmental processes and patterns that surround the diverse floral morphologies are equally diverse among Proteoideae (sensu Johnson and Briggs, 1975). Conserved and taxonomically variable ontogenetic and morphological floral and inflorescence features are discussed below. These include the typology of the racemose inflorescences; the morphogenetic processes of stamens; and the developmental events that produce diverse carpel forms. In addition, the

nectaries among Proteae and Adenanthiinae are morphologically different from the nectaries in other proteaceous flowers (Chapter 2).

Patterns of diversity - Inflorescences - As in other proteaceous taxa, the inflorescences of proteoid taxa are fundamentally racemose in architecture. Diversity among examined taxa includes: proliferation of secondary and in some cases tertiary branches (Symphionema, Synaphea, Stirlingia); reduction of flower numbers on an inflorescence (Adenanthos); and condensation (lack of internode elongation) into heads or capitula (Isopogon, Stirlingia, Serruria) (Venkata Rao, 1971; Johnson and Briggs, 1975; Briggs and Johnson, 1979; Douglas, Chapter 2). In most examined taxa (including most other proteoidean taxa not described here, except for Cenarrhenes and Beauprea that each have axillary racemes) the inflorescences are the product of terminal conversion of the shoot apex into an inflorescence apex (auxotelic). In its most general form, the inflorescence is frondobracteose, there being a serial transformation from leaves to bracts acropetally along the axis. At the other extreme, the inflorescence axis bears only bracts (Adenanthos, Serruria) and forms a non-showy involucre that can become showy in other proteean taxa (Protea, Leucadendron).

Stamen morphogenesis - The morphogenetic events of stamens among examined taxa are developmentally and taxonomically diverse. There are three processes in stamen morphogenesis that are morphologically diverse: 1) connation or fusion between neighbouring filaments and anthers; 2) lateral reduction or partial sterility of anthers and 3) the development of appendicular connectives.

Connation of filaments and anthers - Among all taxa examined, there is fusion of the upper portion of the filament, just proximal to the anthers, between neighboring stamens. Among Conospermeae, except Petrophlinae, the filament connation persists

through anthesis, and the anthers form a tube around the style. In all other taxa examined (Isopogon, Petrophile, Adenanthos and Proteeae), the filaments are connate until the time of anthesis. In the latter groups, the points of fusion on the filaments become lobed and enlarged. In the same taxa, pollen is deposited onto the modified stylar outgrowth or pollen-presenter at anthesis, and the emptied anthers reflex away with the tepals. Connation among the upper portions of the filaments is a character present in all Proteoideae in bud stages; the anthers form a tube around the style.

The anthers of taxa in Conospermeae (except for Petrophilinae) are connate to varying degrees. In Symphionema, Conospermum, Synaphea and Stirlingia, (and Cenarrhenes, Beaupreopsis; not described) the lateral 'margins' of neighbouring anthers become connate. There is an apparent trend of increasing fusion among these taxa: Symphionema stamens are weakly connate at the filaments and tops of the anthers, whereas in the other taxa, the anthers are tightly appressed and they form a common chamber (Venkata Rao, 1971; Johnson and Briggs, 1975). It should be noted that connation is limited to the margins of the anthers. In addition, the microsporangia of neighboring anthers do not become confluent until after pollen maturity. How and when the neighboring anthers separate at anthesis is unknown.

Neighbouring anthers are held together as a tube around the carpel via different forms of fusion among Proteoideae. The presence of anther tubes has been identified in some other angiosperms that have secondary pollen presentation systems (Campanula: Vogel, 1975; Campanula, Jasione, and Lobelia: Erbar and Leins, 1989; Campanulales-Asterales complex: Leins and Erbar, 1990; Acicarpha: Erbar, 1993). In the same taxa, the anthers are held together as a tube around a non-elongate bristle-bearing style (Campanula) or a swollen stigmatic area (Acicarpha) before anthesis. During anthesis, the anthers dehisce, depositing their pollen onto the bristled region of the style which subsequently elongates (Vogel, 1975) whereas in the taxa lacking specialized bristles,

the pollen is deposited as a hood over the summit of the stigma (Erbar, 1993). In Conospermeae examined here (except for species of Petrophilinae), there is no deposition of pollen onto a secondary presentation system. Among the other taxa, the pollen is deposited onto a modified structural outgrowth of the top of the style that is called the pollen-presenter, among Proteaceae. In Isopogon and Petrophile, the pollen-presenter is diametrically broad and has rows of trichomes or bristles that appear to hold the pollen. Among the other taxa, including Adenanthos, Serruria and all other Proteae, there are no bristles or hairs on the usually slightly enlarged club like pollen-presenter.

In other angiosperms with secondary pollen presentation (pollen-presenters), the style elongates and the position of the stigma changes (Erbar and Leins, 1989; Leins and Erbar, 1989). In bud, the stigma is longitudinally positioned at the same level or slightly beneath the anthers. At anthesis, the anthers dehisce, depositing their pollen onto the stigma. The stigma, with the pollen, is then elevated by the elongating style. The secondary pollen presentation systems among proteoidean taxa examined here (Adenanthos, Isopogon, Serruria as well as all other Proteinae; Venkata Rao, 1971; pers. obs.) are not the same as those found by Erbar and Leins (1989) and Leins and Erbar (1990). Prior to anthesis, the slightly broadened pollen-presenter appears to be "clasped" by the anthers, particularly the connate lobes of the distal portions of the filaments. In these taxa, the style elongates, is exserted from the perigon, and forms an arch, but does not affect the position of the pollen-presenter. Thus it appears probable that the function of the connate and enlarged lobes of the upper portion of the filaments is to serve as a "clasping ring" holding the pollen-presenter in place until anthesis.

Lateral reduction of thecae--Partial sterility of anthers via lack of development of one of the two thecae on an anther is termed lateral reduction (Goebel, 1931). Lateral reduction occurs in Conospermum, Synaphea, and Adenanthos obovatus examined here. Lateral reduction of anthers has been found in several angiosperms including

Acanthaceae, Cannaceae, and Labiateae (Trapp, 1956a, b) and in some species of the heterogeneous legume group Cassiinae (Tucker, in press). Besides resulting in highly zygomorphic flowers, lateral reduction has been associated with pollination mechanics in Salvia pratensis (Trapp, 1956a); the sterile half of the anther becomes attenuate and serves as a lever. Attenuate elongation of the sterile theca occurs in Conospermum flowers. Carolin (1961) has suggested that these flowers have explosive pollen dispersal following the contact with a fly or small bee (hence the common name "Smoke-bush"). Detailed examination of pollination syndromes within Conospermum is planned.

There are two different forms of lateral reduction of the anthers in the three taxa examined: positional and temporal. The positional form of lateral reduction is observed in Synaphea and Conospermum. The fertile anther and two fertile thecae are in the adaxial half of the flower in Conospermum, and in the abaxial half of the flowers of Synaphea. The staminode does not become attenuate and or have attenuate laterally reduced thecae in Synaphea. The abaxially positioned staminode in Synaphea becomes fused with the basal portion of the broadly enlarged and clavate stigma. Johnson and Briggs (1975) assert that the opposite direction of zygomorphy in these two taxa is the result of two separate origins.

Temporal lateral reduction occurs relatively late in development of the thecae in the frontally positioned anthers of Adenanthos obovatus compared to that in Conospermum and Synaphea. Lateral reduction in Conospermum and Synaphea begins at the earliest stages of anther differentiation, prior to the formation of the transverse furrow. Such differences in timing suggest that the laterally reduced anthers is a convergence between Adenanthos and the other taxa and probably does not reflect common ancestry. In addition developmental evidence also supports convergence including the initiation of the abaxial stamen (that will become a staminode) before the other stamens in Adenanthos, compared to the normal initiation pattern of laterals first

and the sagittal pair second. In addition, the anthers of Adenanthos do not become laterally fused, and they have a small rounded appendicular connective, common among Proteeae. In Conospermum and Synaphea the thecae overtop the summit of the connective, similar to Stirlingia.

Carpel morphogenesis - *Stigma divergences at tribal levels*- - There are two primary differences among stigma morphologies in the taxa examined. The first difference involves the position of the stigma relative to the summit of the carpel. Among Conospermeae investigated the stigma differentiates terminally on the carpel. In Adenanthos and Serruria (as well as all other Proteae) the stigma is laterally positioned, "oblique" to the summit ("oblique" used by Haber, 1966). The second difference comprises the 'concealment' of the stigma until receptivity. In all Conospermeae, the stigma is generally open at anthesis. In the other taxa, the stigmatic papillae are enveloped by the carpel margins at anthesis which separate slightly at receptivity.

Stigma divergences among Conospermeae- - Several types of stigmas are found among Conospermeae. In Cenarrhenes and Beaupreopsis, the stigma is punctiform. In Symphionema the stigma is capitate to incompletely porate, the margins not fused on the adaxial side. Stirlingia and Synaphea stigmas are large and disk-shaped. The difference between the two is that in the latter, there is no enlargement of the adaxial portion of the stigma, and the staminode is fused to the base of the stigma. Conospermum has a dorsiventral hooded and porate stigma, the product of early fusion and expansion of the top of the carpel. Isopogon and Petrophile both have porate stigmas with small stigmatic papillae.

Nectaries - Douglas (Chapter 2) asserts that the nectaries in proteaceous taxa are functional enations that were probably present in the early ancestor of the family, and

have subsequently diversified in numerous taxonomic lines. This concurs with the hypothesis of Johnson and Briggs (1975). Among taxa examined here, only the Proteae and Adenanthiinae have nectaries (Cennarhenes, Beauprea, and Beaupreopsis also have nectaries). Among all other Conospermeae, the flowers lack receptacular nectaries, and there is no evidence of vestigial or suppressed nectaries based on ontogenetic studies. Bellendena montana (Persoonioideae; Douglas, Chapter 2) also totally lacked nectaries. Venkata Rao (1971) asserts that the flowers of Proteaceae are primitively glandless and that Bellendena, with a diploid chromosome number of 10, represents the basal member of the family from which Symphionema (2n= 20) and other glandless Conospermeae are derived.

Compared to the nectaries of Persoonieae, the nectaries in Proteae and in Adenanthos are morphologically different both in position at initiation and in morphology. In flowers of Persoonieae, four nectary lobes are initiated in alternistamenous positions from an enlarged and flat floral receptacle. Nectary lobes in taxa of Proteoideae examined here are initiated in alternistamenous positions from a concave disk or short hypanthium. In addition in Persoonieae, the nectaries are relatively thick whereas in Proteae and Adenanthos, the nectaries are thin, laminar structures. Anatomically, the nectaries of Proteeae and some species of Adenanthos are not vascularized (Haber, 1959, 1966; Venkata Rao, 1971). Nectaries in Persoonia are vascularized by the floral stele or in Placospermum by the staminal traces. Such differences imply that the nectaries among these taxa might not be homologous or that the nectaries are secondarily derived in Proteae and Adenanthiinae. Considering that Johnson and Briggs (1975) place Persoonieae at the base of the Proteaceae, the different form of the nectaries in Proteoideae and Adenanthos suggest that the latter taxa have secondarily acquired and/or modified the nectary lobes. A phylogenetic analysis of the family will test these theories (Douglas, Chapter 6).

There are similar and contrasting features of flowers and inflorescences among Proteoideae and Persoonioideae (Douglas, Chapter 2). Similarities between flowers of the two families include: the flowers are borne singly within bract axils; the sequential initiation of the floral organs is similar except in Adenanthos species; the organization of the flowers is the same. There are several contrasting features among taxa in the two subfamilies. Contrasting features include inflorescence architecture which in general is loosely organized among Persoonioideae (except Bellendena) compared to the inflorescences among Proteoideae which can show condensation to form heads as well as reduction in flower number, and proliferation of flower number. Compared to Persoonioideae, there is a tendency among proteoidean taxa towards highly zygomorphic flowers via differential growth of the perianth, lateral suppression of the anthers, connation of the stamens, and different types of stigmas. Among Persoonioideae, the flowers are generally actinomorphic and do not have similar elaborations. The flowers of Proteoideae are comparatively more synorganized; the different organs have additive affects on one another. For example, not only do the stamens become connate into a tube around the carpel, but in the more derived taxa, the stamens hold the pollen-presenter in an optimal position for secondary pollen presentation systems. Increasing diversity includes the reduction in size of the filament, a result of zonal growth proximal to the filament/tepal connection and probably associated with pollen-presentation systems.

LITERATURE CITED IN CHAPTER 3

Baum, H. 1951. Vorläuferspitzen im Blütenbereich. Österreich Botanische Zeitschrift 98: 280-291.

Briggs, B. G. and L. A. S. Johnson. 1979. Evolution in the Myrtaceae - Evidence from inflorescence structure. Proceedings of the Linnean Society of New South Wales 102: 157-272.

Carolin, R. 1961. Pollination of the Proteaceae. The Australian Museum Magazine 13: 371-374.

Eames, A. J. 1961. Morphology of the Angiosperms. McGraw-Hill Book Company, Inc. New York.

Erbar, C. and Leins, P. 1989. On the early floral development and the mechanisms of secondary pollen presentation in Campanula, Jasione and Lobelia. Botanische Jahrbücher der Systematik 111: 29-55.

Erbar, C. 1993. Studies on the floral development and pollen presentation in Acicarpha tribuloides with a discussion of the systematic poition of the family Calyceraceae. Botanische Jahrbücher der Systematik 115: 325-250.

Friis, E. M. and P. K. Endress. 1990. Origin and evolution of angiosperm flowers. Advances in Botanical Research 17: 99-162.

Goebel, K. von. 1931. Blütenbildung und Sproszgetaltung. Jena.

Grimes, J. 1992. Metamerism, heterochrony, and inflorescence morphology of the Pithecellobium-complex (Leguminosae: Mimosoideae: Ingeae). Brittonia 44: 140-159.

Haber, J. M. 1959. Comparative anatomy and morphology of flowers and inflorescences of the Proteaceae I. Some Australian taxa. Phytomorphology 9: 325-358.

Haber, J. M. 1961. The comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae II. Some American taxa. Phytomorphology 11: 1-16.

Haber, J. M. 1966 Comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae III. Some African taxa. Phyomorphology 16: 490-527.

Heywood, V. H. 1993. Flowering Plants of the World. New York: Oxford University Press.

Johnson, L. A. S. and B. G. Briggs. 1975. On the Proteaceae--the evolution and classification of a southern family. Botanical Journal of the Linnaean Society 83-182.

Kausik, S. B. 1938. Studies in the Proteaceae. II. Floral anatomy and morphology of Macadamia ternifolia. Proceedings of the Indian Academy of Sciences, 8B: 45-62.

Kausik, S. B. 1941. Studies in the Proteaceae. VI. Vascular anatomy of the flower of Grevillea robusta. Proceedings of the National Institute of Sciences of India 7: 257-266.

Kunze, Von Henning. 1978. Typologie und Morphogenese des Angiospermen-Staubblates. Beiträge zur Biologie der Pflanzen 239 -304.

Leins, P. and Erbar, C. 1990. On the mechanisms of secondary pollen presentation in the Campanulales-Asterales complex. Botanica Acta 103: 87-92.

Nelson, E. C. 1978. A Taxonomic revision of the genus Adenanthos (Proteaceae). Brunonia 1: 303-406.

Rourke, J. P. 1980. The Proteas of Southern Africa. Cape Town: Purnell.

Rourke, J. P. 1984a. A revision of the genus Mimetes Salis. (Proteaceae). Journal of South African Botany, 50: 171-236.

Rourke, J. P. 1984b. Vexatorella Rourke, a new genus of the Proteaceae. Journal of South African Botany, 50: 373-391.

Schacht, H. 1853. Entwicklugnsgeschichte der Blüthe und Frucht von Manglesia cuneata. Bot. Ztg 2: 465-471.

Trapp, A. 1956a. Entwicklungsgeschichtliche Untersuchungen über die Antherengestaltung sympetaler Blüten. Beiträge zur Biologie der Pflanzen 32: 279-312.

Trapp, A. 1956b. Zur Morphologie und Entwicklungsgeschichte der Staubblatter sympetaler Blüten. Bot. Studien 5: Jena.

Tucker, S. C. In press. Stamen structure and development in legumes, with emphasis on poricidal stamens of caesalpinioid tribe Cassieae. *In* W. D'Arcy and R. Keating (eds.) The anther: form, function, and phylogeny. Cambridge University Press.

Venkata Rao, C. 1960. Studies in the Proteaceae I. Tribe Persoonieae. Proceedings of the National Institute of Sciences, India 26B:300-337.

Venkata Rao, C. 1961. Studies in Proteaceae II. Tribes Placospermeae and Conospermeae. Proceedings of the National Institue of Sciences, India 27B126-151.

Venkata Rao, C. 1967. Morphology of the nectary in Proteaceae. New Phytologist 66: 99-107.

Venkata Rao, C. 1971. Proteaceae. Botanical Monograph #6. Council of Scientific and Industrial Research. New Delhi.

Vogel, S. 1975. Campanula rotundifolia (Campanulaceae) Pollination durch Apis mellifica (Hymenoptera) - Mellitophilie. Encyclopaedia cinematographica E 2049.

Weberling, F. 1989. Morphology of Flowers and Inflorescences. Cambridge University Press. Great Britain.

Wrigley, J. W. and M. Fagg. 1988. Banksias, Waratahs and Grevilleas. Collins Publishers. Sydney, N.S.W., Australia.

CHAPTER 4

INFLORESCENCE ONTOGENY AND FLORAL ORGANOGENESIS IN GREVILLEOIDEAE (PROTEACEAE), WITH EMPHASIS ON THE NATURE OF THE FLOWER PAIRS

INTRODUCTION

Mature flowers of the proteaceous subfamily Grevilleoideae (approximately 42 genera) are not arranged in a clear and simple relation to an axis as they are in the racemes of the other four subfamilies (Venkata Rao, 1957 et seq.; Johnson and Briggs, 1963, 1975). Within the other four subfamilies of Proteaceae (Persoonioideae--four genera sensu Weston, 1983; Proteoideae--23 genera; Sphalmioideae--monotypic; Carnarvonioideae--two species) a single flower develops in the axil of a bract or leaf. In all taxa of Grevilleoideae, inflorescences are composed of sets of paired flowers subtended by a "common bract" (Engler, 1894; Johnson and Briggs, 1963, 1975). The presence of flower pairs is exclusive to Grevilleoideae (Johnson and Briggs, 1975). Engler (1894) used the presence of flower-pairs to modify the original classification of the then two proteaceous subfamilies by Brown (1810) who had relied on fruit characters (indehiscent vs. dehiscent).

Location of the sagittal and frontal planes of grevilleoid flowers is often based on the position of the common bract and the primary inflorescence. Payer (1857) referred to different sides of Grevillea thelemannia flowers as posterior and anterior; the posterior side being closest to the common bract and the anterior side being closest to the primary inflorescence axis. In flowers of the other four subfamilies, the four tepals are arranged in pairs in the sagittal and frontal planes; four stamens are present, each one superposed to a tepal, and the carpel cleft faces the adaxial tepal.

Several variable floral features among grevilleoid genera make assessments of floral planes difficult from studies of mature flowers: 1) the presence or absence of individual floral bracts subtending each flower of a pair; 2) in some genera, the tepals and stamens can appear diagonal (skewed) relative to the common bract (Fig. 4.3); 3) depending on the taxon, the flower pairs can be either sessile or share a common stalk or

peduncle; and 4) in different genera, the orientation of the carpel can be either dorsiventral or diagonal relative to the other floral organs (Venkata Rao, 1957, 1971; Haber, 1959, 1961, 1966; Johnson and Briggs, 1963, 1975). The combination of these four features, in addition to the presence of flower-pairs, have proven to be limiting if not confounding factors hindering establishment of homology in floral organization among grevilleoid taxa.

The ultimate goal of this paper is to analyze the diverse orientations of flowers among Grevilleoideae in terms of three interconnected fundamental components. The first component is the developmental events producing the flower pairs, including an evaluation of the conflicting hypotheses about the phylogenetic origin of the flower pairs. Conflicting hypotheses include: reduced triads (dichasia) via loss of the terminal flower (Haber, 1959, 1961, 1966); multiple reductions of secondary, tertiary and quaternary racemose inflorescence branches (Venkata Rao, 1971); and reduction of secondary racemose inflorescence axes (indeterminate paniculate florescence branches; Johnson and Briggs, 1975). The second component involves the early organogenetic stages of the flowers. The third component is the skewed position of flowers in some taxa in relation to the common bracts. The evidence obtained from this study is part of a broad comprehensive study of comparative floral development in Proteaceae. The aim of these studies is to define the developmental events associatied with taxonomic diversity in the family as well as to clarify structural homologies among the taxonomically distinct flowers. This study will provide ontogenetically derived concepts of homology (organizational similarities) among proteaceous flowers that will be ultimately examined in a phylogenetic context.

METHODS AND MATERIALS

Taxa and provenance included in the analysis are listed in Table 4.1. All plant material was fixed in formalin-acetic acid-ethanol (FAA - 5 ml (37%) formalin - 5 ml

glacial - 90 ml 50% ethanol), and subsequently stored in 70% ethanol. Floral material was microdissected in 95% ethanol under a Wild dissecting microscope with fiber optic illumination. Dissected materials were dehydrated through an acetone series and critical point dried with a Denton apparatus in carbon dioxide. Dried material was affixed to aluminum (SEM) stubs with Photo-Mount glue (3M, Inc.) or with colloidal graphite (Pelco inc.). The material was then coated with 100-500 Angstroms of gold-palladium in a Hummer II sputter coater. Coated materials were examined with a Cambridge S-260 scanning electron microscope at 20 or 25 kV. Images were recorded on Kodak Tri-X Pan 4x5 film and later printed on Kodak Polycontrast Rapid III R.C. paper.

Terminology - Floral planes - (Fig. 4.1) - There are different terms associated with the different planes in flowers and structures. Terminology that will be used include the median **sagittal** plane that bisects a flower, its subtending leaf (or bract) and the inflorescence axis into two mirror-image halves. The median **frontal** plane is perpendicular to the median sagittal plane. The same two terms are used to describe the short-shoot meristems in grevilleoid flower ontogeny (see Fig. 4.2). The **adaxial** side of the flower is the upper half, closest to the inflorescence axis, and the **abaxial** side is the lower half of the flower closest to the subtending leaf (or bract). There are different terms associated with the symmetry of flowers. **Actinomorphy** is synonymous with radial symmetry, in which the flower has multiple planes of symmetry; **bisymmetry** describes structures that have two planes of symmetry, in which each bisects the structure or flower into two mirror-image halves (Weberling, 1989; Friis and Endress, 1990); and **zygomorphy (or dorsiventral symmetry)** describes structures or flowers that have one plane of symmetry; the abaxial and adaxial halves are not mirror-images (Weberling, 1989; Friis and Endress, 1990).

Table 4.1. Species examined and provenance. ** = species examined but not illustrated.

Taxa	Native to:	Source
Banksia pulchella R. Br.	S.W. Australia	University of California at Santa Cruz (U.C.S.C.) Arboretum.
Austromuellera trinerva C.T. White	N.E. Queensland	N.E. Queensland
Darlingia darlingiana (F. Muell.) L. Johnson	N.E. Queensland	N.E. Queensland; T. Irvine's private arboretum
Eucarpha deplanchii Brongn. and Gris.	New Caledonia	New Caledonia (P. Weston, coll.)
Cardwellia sublimis F. Muell.	E. Queensland	N.E. Queensland: 20 km NW of Atherton: Mt. Spec, Qld.
Orites revoluta R. Br.	Tasmania	Tasmania (J. Chappill, coll.)
Lambertia inermis R. Br.	S. W. Australiaa	U.C.S.C. Arboretum
Virotia leptophylla (Guillaumin) L. Johnson and B. Briggs	New Caledonia	New Caledonia (P. Weston, coll.)
Brabejum stellatifolium L.	South Africa	Kirstenbosch Gardens gardens, South Africa, (T. McLellan; D. Keats; colls.)
Macadamia integrifolia Maiden and Betche	E. Australia	Royal Botanic Gardens (R.B.G.) Sydney; U.C.S.C. arboretum; University of Melbourne; T. Irvine's private arboretum, Atherton; Waimea Gardens, Hawaii (E.M. Harris, coll.)
Hollandaea sayerana (F. Muell.) L.S. Smith	N.E. Queensland	N.E. Queensland (B. Hyland, coll.)
Opisthiolepis heterophylla L.S. Smith	N.E. Queensland	N.E. Queensland, T. Irvine's private arboretum
Stenocarpus salignus R. Br.	Eastern and N.E. Australia	Strybing arboretum, San Francisco, California.
Lomatia fraxinifolia R. Br.	E. Australia	Strybing Arboretum
Telopea mongaensis Cheel.	S.E. Australia	R.B.G. Sydney (P.Weston, coll.)
Telopea speciosissima (Sm.) R. Br.	E. Australia	U.C.S.C. Arboretum
Embothrium coccineum Forst.	Chile, South America	U.C.S.C. Arboretum; R.B.G. Sydney (P. Weston, coll.)
Grevillea baileyana McGillivray	N.E. Queensland	N.E. Queensland

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Taxa	Native to:	Source
Grevillea glabella R. Br.	S.E. Australia	U.C.S.C. Arboretum
Grevillea wilsonii A. Cunn.	W. Australia	U.C.S.C. Arboretum
**Orites excelsa R. Br.	N.E. Queensland	N.E. Queensland (B. Hyland, coll.)
** Neorites kevediana L.S. Smith	N.E. Queensland	N.E. Queensland, B.Gray (coll)
** Knightia excelsa R.Br.	New Zealand	U.C.S.C. Arboretum
** Darlingia ferruginea J.F. Bailey	N.E. Queensland	Atherton, N.E. Queensland
** Banksia praemorsa Andrews	S.W. Australia	U.C.S.C. Arboretum
** Banksia ericifolia A.S. George	S.W. Australia	U.C.S.C. Arboretum
** Musgravea stenostachys L.S. Smith	N.E. Queensland	N.E. Queensland
** Buckinghamia celsissima F. Muell.	E. Queensland	James Cook University Arboretum, (Peter Jobson coll.)
** Bleasdalea bleasdalei (Syn. Turillia) (F. Muell.) A.C. Smith	Eastern Queensland	Eastern Queensland, Mt. Spec.
** Gevuina avellana Molina	Chile, South America	U.C.S.C. Arboretum
** Lambertia formosa Sm.	S. W. Australia	U.C.S.C. Arboretum
**Roupala montana Aubl.	South and Central America	French Guiana, South America (E.M. Harris and M.F. Quigley, coll.); Costa Rica (M. Wiemann, coll.)
**Macadamia tetraphylla L.A.S. Johnson	E. Australia	R.B.G. Sydney; R.B.G. Melbourne; T. Irvine's private arboretum, Atherton.
** Athertonia diversifolia (C.T. White) L.S. Johnson and B. Briggs	N.E. Queensland	N.E. Queensland (B. Hyland, coll.)
** Helicia lamingtonia (F.M. Bail.) C.T. White	N. E. Queensland	N.E. Queensland (B. Gray, coll.)
** Stenocarpus davallioides D.F. Foreman and B.P.M. Hyland	N.E. Queensland	Atherton, N.E. Queensland, Tony Irvine's Private Arboretum
** Alloxylon flammeum P.H. Weston and M.D. Crisp	N.E. Queensland	Atherton, N.E. Queensland

con'd

Taxa	Native to:	Source
** Oreocallis brachycarpa (Sleum.) Sleum.	New Guinea	R.B.G. Sydney (Weston, coll.)
** Hakea elliptica R. Br.	W. Australia	U.C.S.C. Arboretum
** Grevillea sericeae R. Br.	W. Australia	U.C.S.C. Arboretum
** Grevillea vestita (Endl.) Meissner	S.W. Australia	U.C.S.C. arboretum
**Sleumerodendron austro-caledonicum (Brongn. and Gris) Virot	New Caledonia	New Caledonia (P. Weston coll.)

Inflorescence terminology - Inflorescence terminology follows the definitions by Briggs and Johnson (1979), Weberling (1989), and Grimes (1992). **Principal axis** refers to the primary or main floriferous axis. Among Grevilleoideae, the first-order bract (sensu Abbe, 1974), a leaf homologue or pherophyll (Briggs and Johnson, 1979), is referred to as a **common bract** because it is shared by two flowers. It could be equally referred to as a first-order bract (sensu Abbe, 1974). **Floral bract** refers to the leaf homologue that subtends an individual flower. The term bracteole has been associated with the individual flowers in a pair by several authors. It is not used here because the term is more appropriately used among determinate or anthotelic inflorescences, or in the cases where the 'bracteoles' do not have an axillary bud (MacDonald, 1971, Briggs and Johnson, 1979; Weberling, 1989), or when they are a product of the floral meristem as in the 'pseudoracemes' of some papilionoid legumes (Tucker, 1987a). A **blastotelic** inflorescence is indeterminate compared to an **anthotelic** inflorescence that terminates with a flower (polytelic vs. monotelic sensu Weberling, 1989). Inflorescences develop in leaf axils or can be a modified extension of the terminal vegetative axis. Auxotely and anauxotely are terms generally associated with the growth of the shoot in relation to the position of blastotelic inflorescences. In an **anauxotelic** shoot, growth continues beyond the region of flowering or inflorescence development; **auxotelic** shoots terminate with the development of an inflorescence (Briggs and Johnson, 1979; Weston, 1983; Grimes, 1992). Troll (in Weberling, 1989) distinguished two types of compound racemose inflorescences related to the secondary inflorescence branch axes or partial inflorescences. **Homothetic compound racemes** are composed of only lateral racemes that are partial inflorescences; **heterothetic compound racemes** are composed of lateral racemes and a terminal raceme (Fig. 4.135; Weberling, 1989). Briggs and Johnson (1979) refer to secondary inflorescence branches or partial inflorescences as **uniflorescences,** and qualify the term with the adjectives racemiform,

thyrsiform etc... Briggs and Johnson (1979) use **conflorescence** as a term for inflorescences made up of uniflorescences (secondary branches).

Inflorescence diversity among Proteaceae - To discuss the possible origin(s) of the flower-pairs, it is necessary to understand inflorescence diversity among Proteaceae. An extensive typological comparison of mature proteaceous inflorescence diversity was reported by Venkata Rao (1965, 1971). All proteaceous inflorescences are blastotelic and primitively racemose (Briggs and Johnson, 1979) or indeterminate (polytelic sensu Weberling, 1989). In almost all inflorescences of Proteaceae, the apex of the principal axis senesces and trichomes can differentiate on it. Trichomes have been observed to differentiate from the residuum in several other angiosperms like Saururaceae (Tucker, 1979) and papilionoid legumes (Hole and Hardwick, 1976; Tucker, 1987a; Tucker and Stirton, 1991). The positions of the flowers and inflorescences vary among taxa. In most species of Persooniinae (Persoonioideae), the relative growth unit of the plant is anauxotelic and single flowers develop in the axils of vegetative leaves or serially reduced leaves (flowering branches sensu Weberling, 1989). In other proteaceous taxa, there are distinct inflorescences. Both anauxotelic and auxotelic shoots are present in the subfamilies Persoonioideae, Proteoideae and Grevilleoideae. In some species, both types occur on the same plant. Most Proteoideae, Bellendena and grevilleoid taxa have auxotelic inflorescences, the terminal portion of the growth unit or shoot developing as an inflorescence (simple or heterothetic). Axillary inflorescences are found in some Proteoideae (Adenanthos obovatus, Nelson, 1978; Leucospermum, in part, Rourke, 1984a; Mimetes, Rourke, 1984b) and in most taxa of Macadamieae in Grevilleoideae (Venkata Rao, 1965). It should be noted that in Placospermum coriaceum (Persoonioideae), grevilleoid taxa (Cardwellia sublimis, Alloxylon flammeum, Orites revoluta) and Carnarvonia araliifolia (Carnarvonioideae) both axillary inflorescences and a terminal inflorescence can be present on the same shoot or growth unit (foliose

heterothetic inflorescences). Inflorescence morphologies are extremely diverse among Proteaceae and will be the focus of another study.

Floral groundplan - (Fig. 4.1) - With rare exceptions, flowers of Proteaceae are four-merous. The flowers are composed of a single whorl of four valvate tepals; the marginal epidermis of each tepal interlocks with the margin of the neighbouring tepal. The term tepals is used because the phylogenetic origin of the single perianth whorl in Proteaceae is uncertain although Proteaceae is hypothetically derived from a monochlamydeous ancestor (pre-Rosidae, Johnson and Briggs, 1975; Douglas, Chapter 2). Each flower has four stamens, one positioned opposite each tepal (superposed or **antetepalous**). The stamens are composed of tetrasporangiate anthers and a basifixed filament. The latter of is generally adnate (via zonal growth) to the opposing tepal lobe (**epitepalous**). Internal to the stamens, a single carpel is present. The orientation of the carpel cleft is unusually varied among taxa of Grevilleoideae and will be the focus of another paper (Chapter 5). In the other four subfamilies, the carpel cleft faces the adaxial tepal (Fig. 4.1; Chapters 2 and 3).

General organography of Grevilleoideae - (Fig. 4.2) - In virtually all examined taxa, two flowers are present in the axil of each common bract (Fig. 4.2D-E). There are numerous common bracts on a principal inflorescence axis (conflorescence sensu Briggs and Johnson, 1979). The two flowers of a pair are initiated from a single second-order meristem initiated in each common bract axil (Fig. 4.2A). The axillary meristem is referred to as a **short-shoot meristem** although it could equally be referred to as a first-order axillary meristem (Abbe, 1974), a brachyblast primordium, or a common meristem. Each flower of a pair, including the individual floral bracts if present (* in Fig. 4.2B-E), is a mirror image of its sister flower, both morphologically and developmentally. Developmentally, each flower is dorsiventrally aligned, usually in relation to a floral bract, so that the median sagittal axis bisects the adaxial and abaxial tepals (Fig. 4.2D-E).

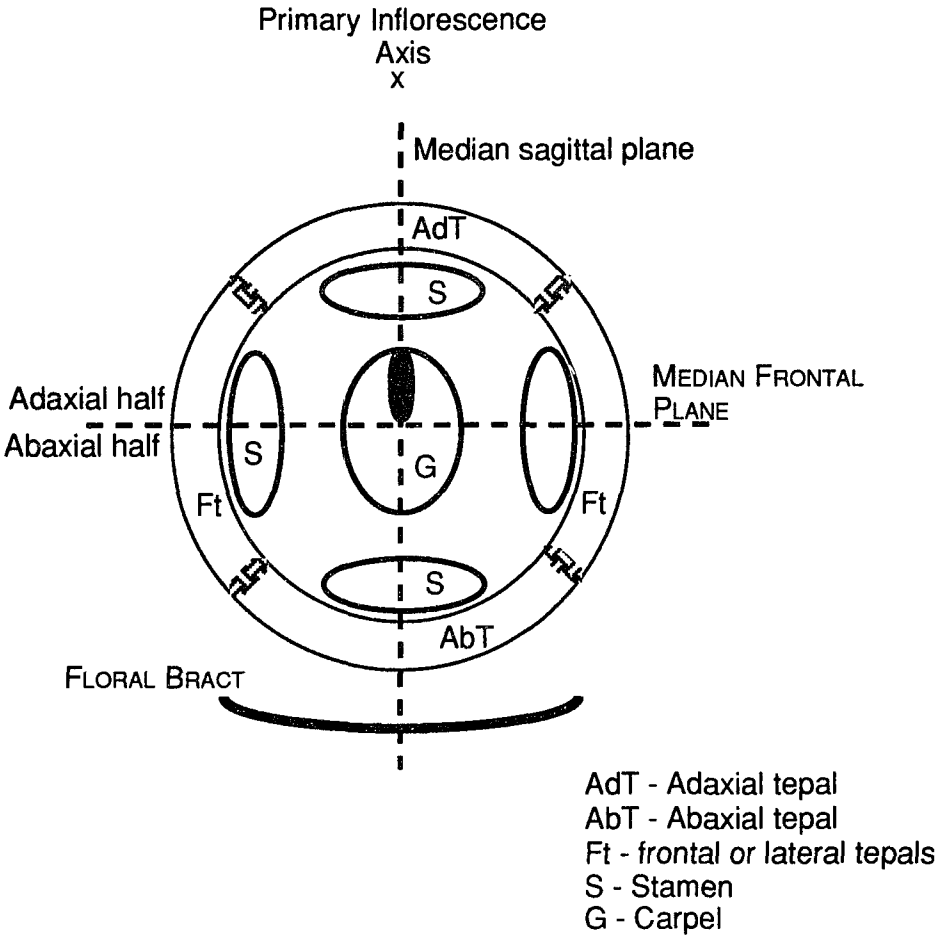


Figure 4.1. Floral diagram of typical proteaceous flower illustrating the numbers and arrangement of floral organs and the various sides of the flower.

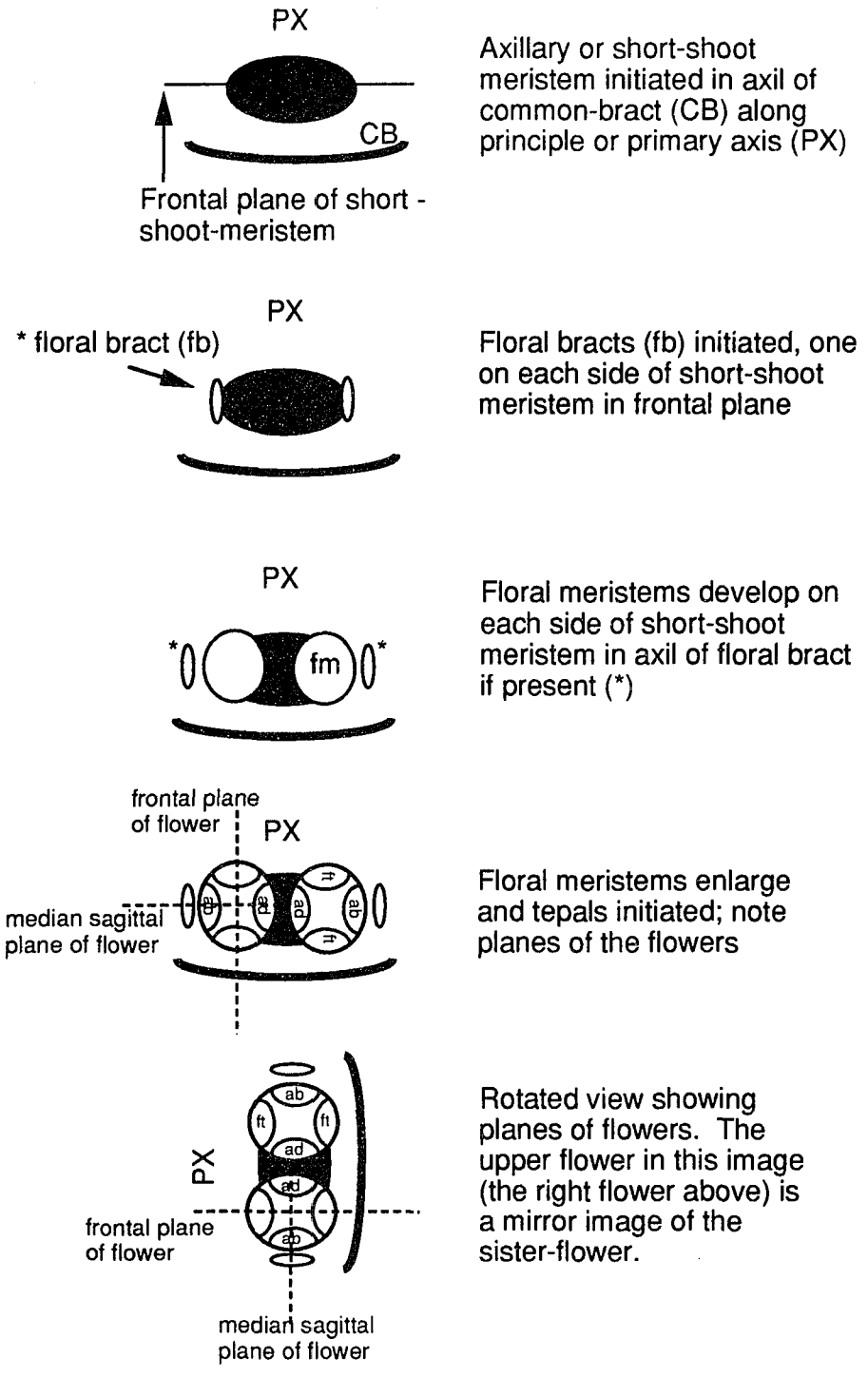
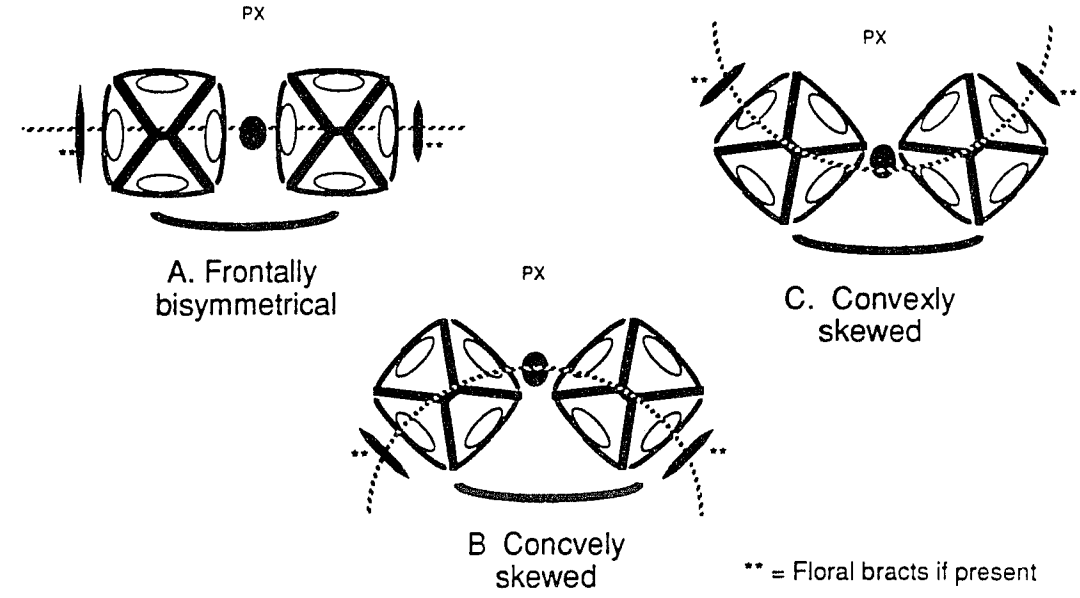


Figure 4.2. Diagrammatic representation of the ontogenetic events involved with the initiation and development of the flower pairs of Grevilleoideae from a short-shoot meristem. Terms used to define the orientations are included.

The orientation of the flowers in a pair can be **skewed (concave or convex)** or **frontally bisymmetrical** in relation to the principal axis and the common bract (Fig. 4.3). These terms are used solely to describe the orientation of *flowers in pairs* and does not define the orientation of a flower in relation to its floral bract. In **frontally bisymmetrical flower pairs** (Fig. 4.3A), a straight line can be drawn that bisects the two floral bracts, the longitudinal axis of the flowers (median sagittal plane), and the apical residuum between the two flowers. In **skewed floral pairs** (Figs. 4.3B-C), the line that bisects the floral bracts, the longitudinal axis of the flowers (median sagittal plane) and the apical residuum is curved either in **concave** (Fig. 4.3B) or **convex** (Fig. 4.3C) alignments in relation to the subtending common bract. These terms are used for the early stages of floral development, usually through aestivation. In later stages, the flowers can become skewed as the axes enlarge and/or the pedicels twist. Such late-stage events will be the topic of other studies.

Organogenesis patterns among flowers of Grevilleoideae are similar to the organogenesis patterns of flowers in Persoonioideae and Proteoideae. Tepal organogenesis of the flowers in Grevilleoideae is similar to that of the flowers in the other Persoonioideae and Proteoideae, although the sequential initiation of tepals in some taxa is difficult to determine due to compression of the floral meristems by the common bracts and/or primary inflorescence axis. Stamen initiation is similar among proteaceous flowers although very difficult to observe in flowers of Grevilleoideae due to what appears to be precocious epitepally. During the dissections, removal of the incurved tepal primordia also resulted in the excision of the anther primordia and/or portions of the remaining floral meristem. Therefore, the initiation of the stamen primordia will be reported at a later date upon more complete investigations.

Aestivation- - (Fig. 4.3D-F) - Aestivation patterns of the tepals among taxa of Grevilleoideae are generally similar to those in flowers of other taxa in the family. The



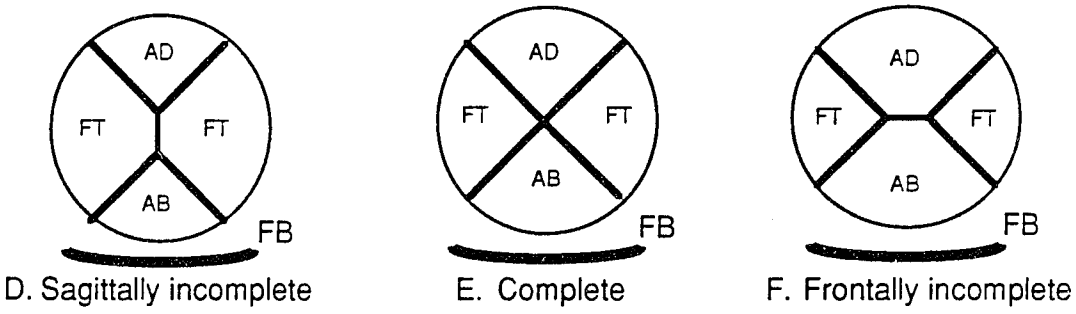


Figure 4.3. **A-C**. Position or skewedness of flowers in a pair in relation to the common-bract. **D-F** Aestivation patterns among tepals in flowers of Proteaceae. FT= frontal tepal; AD= adaxial tepal; AB=abaxial tepal.

two frontal tepal tips converge over the floral meristem (and organs if present) and come into contact with one another, so that tthe sagittal tepals do not meet; the condition is called **sagittally incompletely valvate** (Fig. 4.3D). Variation of aestivation patterns includes: 1) all four tepals converging and coming into contact (**completely valvate**; Fig. 4.3E); 2) the sagittal tepals coming into contact with one another with the frontal tepals positioned in the spaces formed by the sagittal tepals but not meeting, a condition termed **frontally incomplete valvate** (Fig. 4.3F). The latter two are generally uncommon and were seldom found on all flowers of an inflorescence (see below).

OBSERVATIONS

A total of 50 species in 35 genera of Grevilleoideae have been investigated. The earliest developmental stages were available in 35 species and 21 genera. The ontogenies of representative members of tribes and subtribes of Grevilleoideae are described below. Because there were similar patterns expressed by numerous taxa, the earliest stages of floral pair development and tepal organogenesis are summarized for representative speices under tribal and subtribal rankings (sensu Johnson and Briggs, 1975). A total of 16 genera and species are described here. Some sampling limitations resulted in incomplete stages for certain taxa.

Banksieae (Two subtribes: Figs. 4.4-18) - **Banksiinae** - Banksiinae is composed of two speciose australian genera, Banksia and Dryandra. Banksia pulchella is described below as a representative of Banksiinae.

Banksia pulchella - (Figs. 4.4-12) - Along the terminal (auxotelic) inflorescence axis (Fig. 4.4), numerous (>150) common bract primordia are initiated from a broad inflorescence apex (Fig. 4.5). A frontally broad meristem develops within the axil of each common bract (Fig. 4.6). It becomes a short-shoot meristem that subsequently enlarges and becomes arcuate (Fig. 4.7; abaxially concave in Fig. 4.3A). Two floral

Figs. 4.4-12. Flower pair development and floral organogenesis in Banksia pulchella. In all images the common bract (**CB**) is at the bottom. **4**. Early development of principal auxotelic inflorescence axis (arrow) surrounded by young leaves. **5**. Young inflorescence axis initiating common bracts. **6.** Initiation of short-shoot meristems in common bract axils. **7.** Side view of short-shoot meristem becoming concavely skewed. **8**. Polar view of short-shoot; the floral bracts (**arrow on left, FB on right**) have been initiated, and the floral meristems are beginning to enlarge in each floral bract axil. Trichomes develop on the short-shoot residuum (**R**). **9**. Oblique view of left flower of flower pair. The floral meristem has enlarged and trichomes differentiate on the tip of the bract (arrow). **10**. Oblique view of flower pair initiating tepals. The right flower has initiated one of the frontal tepals (**fT**). All four tepals have been initiated on the left flower including the abaxial and adaxial tepals. **11**. Polar view of left flower of pair showing the enlargement of the tepals over the floral meristem. **12.** Polar view of flower pair. The right flower illustrates the sagittally incomplete valvate pattern of aestivation. Tepals are removed in the left flower. **Scale bars:** 4, 5 = 100μm; 6-9 = 25μm; 10-12 = 50μm.

In all subsequent images symbols are C=common bract; FB(also fb)=floral bract; fT=frontal tepal; ad=adaxial tepal; ab=abaxial tepal; R=residuum; PX=primary inflorescence axis; MSP=median sagittal plane and is generally accompanied by an arrow indicating the plane; S=stamen.


4
LF
5
6
CB
CB
7
CB
8
FB
CB
FB
CB
10
fT
adT
fT
abT
fT
CB
11
fT
abT
FB
CB
12
adT
fT
fT
abT
FB
CB

Figs. 4.4-4.12

bract primordia are initiated from the lateral flanks of the short-shoot meristem and are abaxially deflected from the frontal plane (Fig. 4.8). A floral meristem develops within each floral bract axil (Fig. 4.9). Trichomes differentiate on the apical residuum of the short-shoot meristem (Fig. 4.8).

Initiation of the tepals appears rapid; the tepals on each side in the frontal plane appear first ('fT' on the right flower in Fig. 4.10) followed by the initiation of the tepals in adaxial and the abaxial positions in the sagittal plane ('s' arrows in the left flower in Fig. 4.10). The tepals enlarge and converge over the floral meristem and developing organs (Fig. 4.11), and the lateral tepal tips come into contact with one another (Fig. 4.12); the sagittal tepals do not touch one another (Fig. 4.12). Thus, the tepal aestivation pattern is sagittally-incomplete valvation (Fig. 4.3D). The flower pairs do not have elongate peduncles and the individual floral bracts are persistent, becoming peltate in shape later in ontogeny (not illustrated). The flower-pairs are concavely skewed (Fig. 4.3B).

Patterns of short-shoot meristem development similar to those of B. pulchella were observed in B. praemorsa, B. ericifolia (pers. obs) and B. menziesii and B. coccinea by Fuss and Sedgley (1990). It should be noted that the primary flowering axis or spadix of Banksia species examined is anauxotelic (terminal). The taxa of Dryandra vary between axillary compressed heads or capitula, and terminal capitula (George, 1984; Carr, 1984).

Musgraveinae (two genera) - Austromuellera trinerva C.T. White - (Figs. 4.13-18) - An ontogeny of the short-shoot meristem and of the flower pairs similar to that of Banksia species was observed in Austromuellera. Racemes with little elongation of internodes between the common bracts develop in axils of leaf or leaf-scars. From the principal inflorescence apical meristem, hundreds of common bract primordia are initiated (Fig. 4.13) acropetally. Within each common bract axil, a frontally oblate short-shoot meristem is initiated and enlarges (Fig. 4.14 and upper meristem in Fig. 4.15). The short-

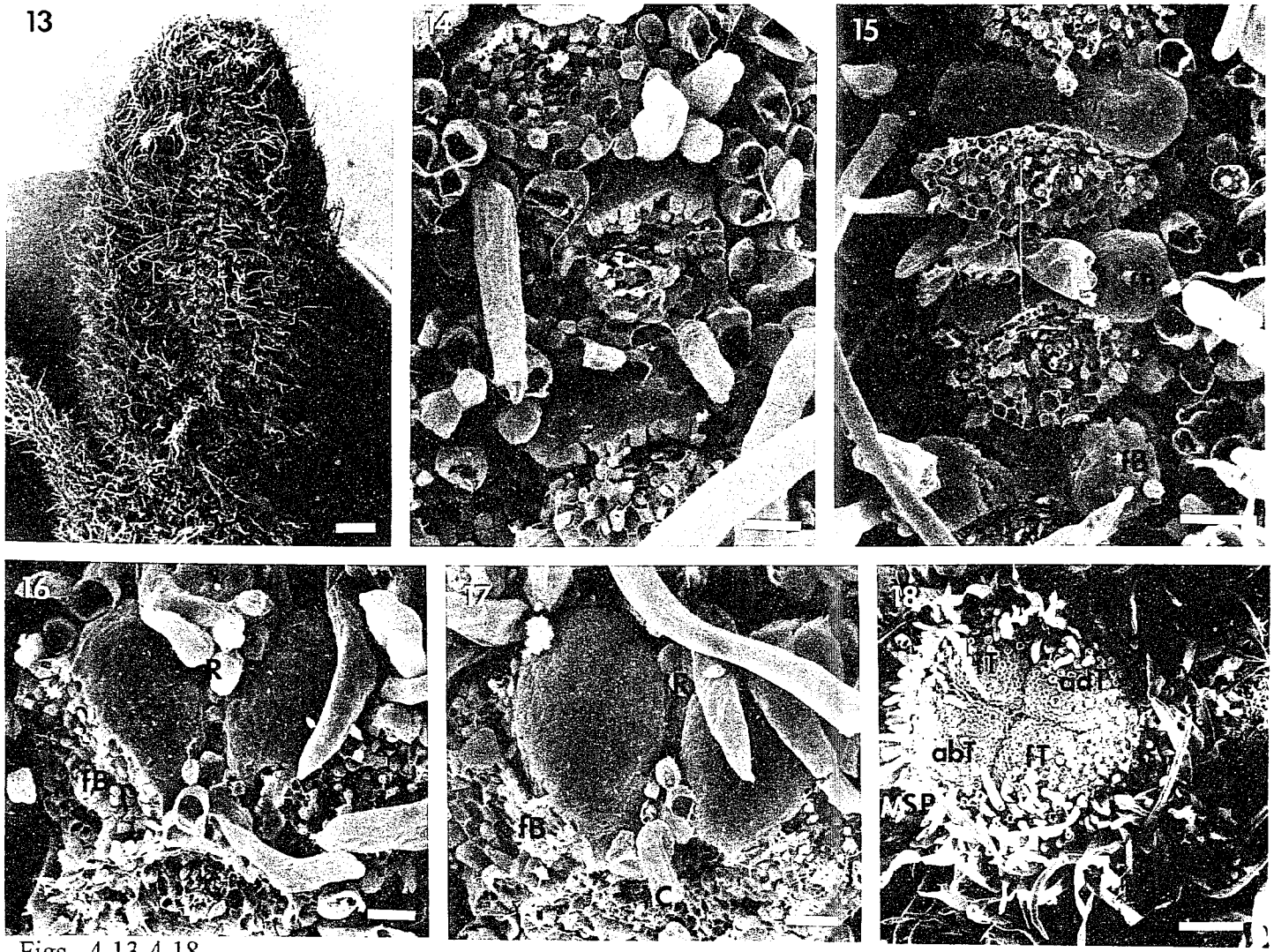
shoot meristems becomes abaxially arcuate in relation to the common bract (Fig. 4.15). Two floral bract primordia are initiated from the arms of the concavely arcuate short-shoot meristem (arrows in Fig. 4.3593) and are deflected abaxially from the frontal plane (bottom in Fig. 4.15). A floral meristem enlarges in the axil of each of the floral bracts (floral bracts removed in Figs. 4.16, 17) resulting in a flower pair. Stages of tepal initiation were not available. After aestivation, the lateral tepals come into contact and the sagittal tepals do not touch one another (Fig. 4.18). The individual floral bracts are persistent. The flower-pairs are concavely skewed (Fig. 4.3B).

Musgravea stenostachya has a developmental pattern similar to that of Austromuellera both in the sagittally incomplete valvate pattern of aestivation and in having concavely skewed flower pairs.

Knightieae (Two subtribes) (Figs. 4.19-39) - **Knightiinae** (Figs. 4.19-30) - All three genera in Knightiinae have been investigated and developmental stages of two are illustrated.

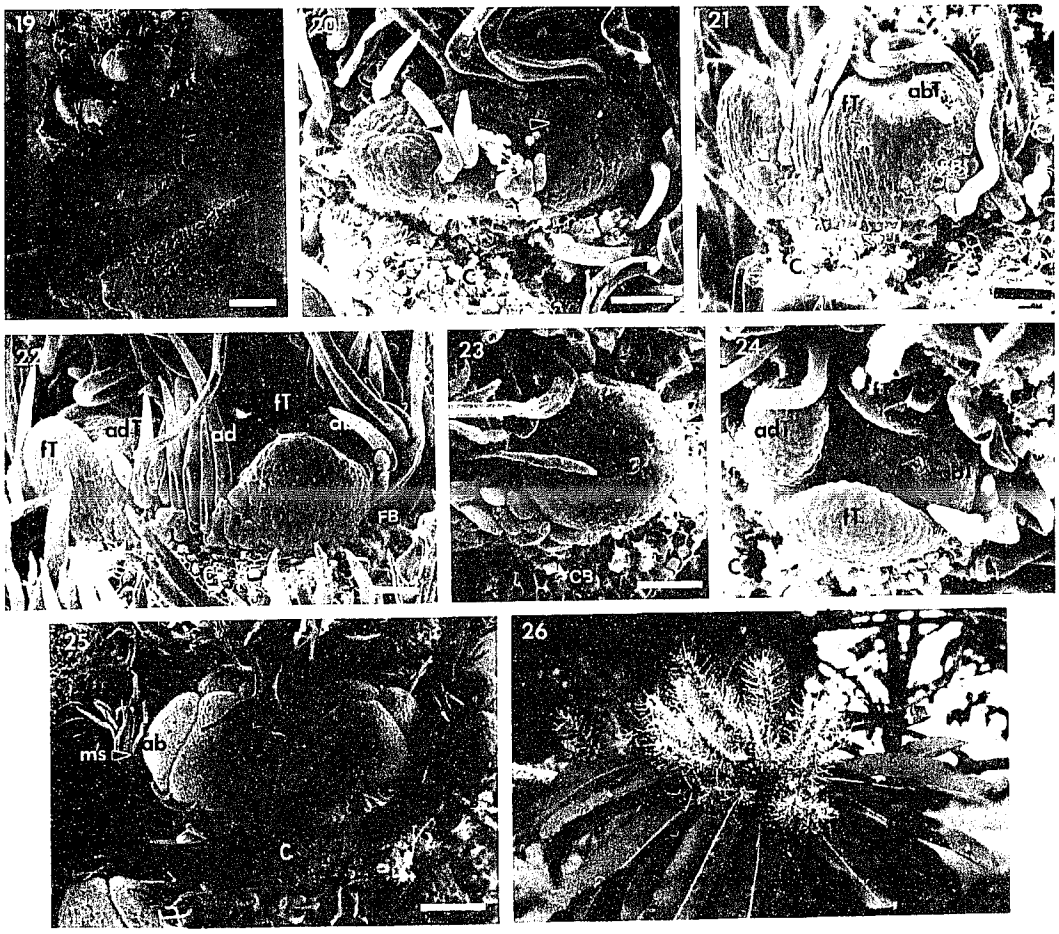
Darlingia darlingiana - (one of two species in genus) (Figs. 4.19-26) - The inflorescence of Darlingia darlingiana (Fig. 4.26) is heterothetic and auxotelic with partial inflorescence axes arising from leaf and bract axils along the axis that terminates with a partial inflorescence. Numerous flowers are present on the principal axis (Fig. 4.19). A frontally oblate meristem is initiated in the axil of each of the common bracts that becomes a short-shoot meristem. A floral meristem enlarges laterally on each side of each short-shoot meristem (Fig. 4.20). As the floral meristems enlarge, trichomes develop in the position where one would expect to find a floral bract (arrow in 21 and 22). Trichomes also differentiate on the apical residuum between the floral meristems (Figs. 4.20-22). The first tepal is initiated laterally from the floral meristem closest to the common bract (Fig. 4.23). The opposite lateral tepal is initiated second, followed by the initiation of the

Figs. 4.13-18. Flower pair development and floral meristem enlargement in Austromuellera trinerva. In all images common bracts (**CB**) are at the bottom. **13.** Young primary inflorescence axis bearing numerous common bracts, some of which have been removed. **14.** Initiation of two short-shoot meristems, each in an axil of a common bracts (removed). **15.** Sequential stages of enlargement of the short-shoot meristem. At the top, the short-shoot meristem becomes arcuate during enlargement. In the middle of the image, the floral bract has been initiated, and in the lower image, trichomes are differentiating on the floral bracts. **16.** Polar oblique view of a short-shoot showing the enlargement of each of two floral meristems in the floral bract (removed) axils. Trichomes differentiate on the short-shoot residuum. **17.** Further enlargement of floral meristems. **18.** Polar image of left flower of a pair showing the sagittally incomplete valvate pattern of aestivation. **Scale bars**: 13 = 500μm; 14, 16, 17 = 25μm; 15 = 50μm; 18 = 100μm.



Figs. 4.13-4.18

Figs. 4.19-26. Flower pair development and floral organogenesis in Darlingia darlingiana. **19.** Young inflorescence axis. **20.** Two floral meristems have been initiated, one from each side of the short-shoot meristem (arrowheads). **21.** Side view of flower pair and the abaxial view of the right flower of the pair showing the trichomes and slight enlargement of the floral bracts. Trichomes are differentiating from the residuum. **22.** Frontal view of flower pair showing tepal initiation on the floral meristems. The lateral frontal tepals are larger than the sagittal tepals. **23.** Polar view of right flower of a pair showing the initiation of the first tepal, the lateral tepal closest to the common bract. **24.** Polar view of right flower of a pair showing the sequential initiation of the sagittal tepals; the adaxial tepal is initiated prior to the abaxial tepal. **25.** Oblique polar view of flower pair showing the incomplete sagittal aestivation pattern of tepals and the arrangement of the flowers in a bisymmetrical pattern relative to the common bract. **26.** Mature inflorescences showing the terminal nature and secondary inflorescence branching of the inflorescence. At anthesis, each axis resembles a bottle-brush and is actinomorphic. **Scale bars**: 19 = 500μm; 20-25 = 50μm. In 26, each inflorescence is approximately 12-20 cm.



Figs. 4.19-4.26

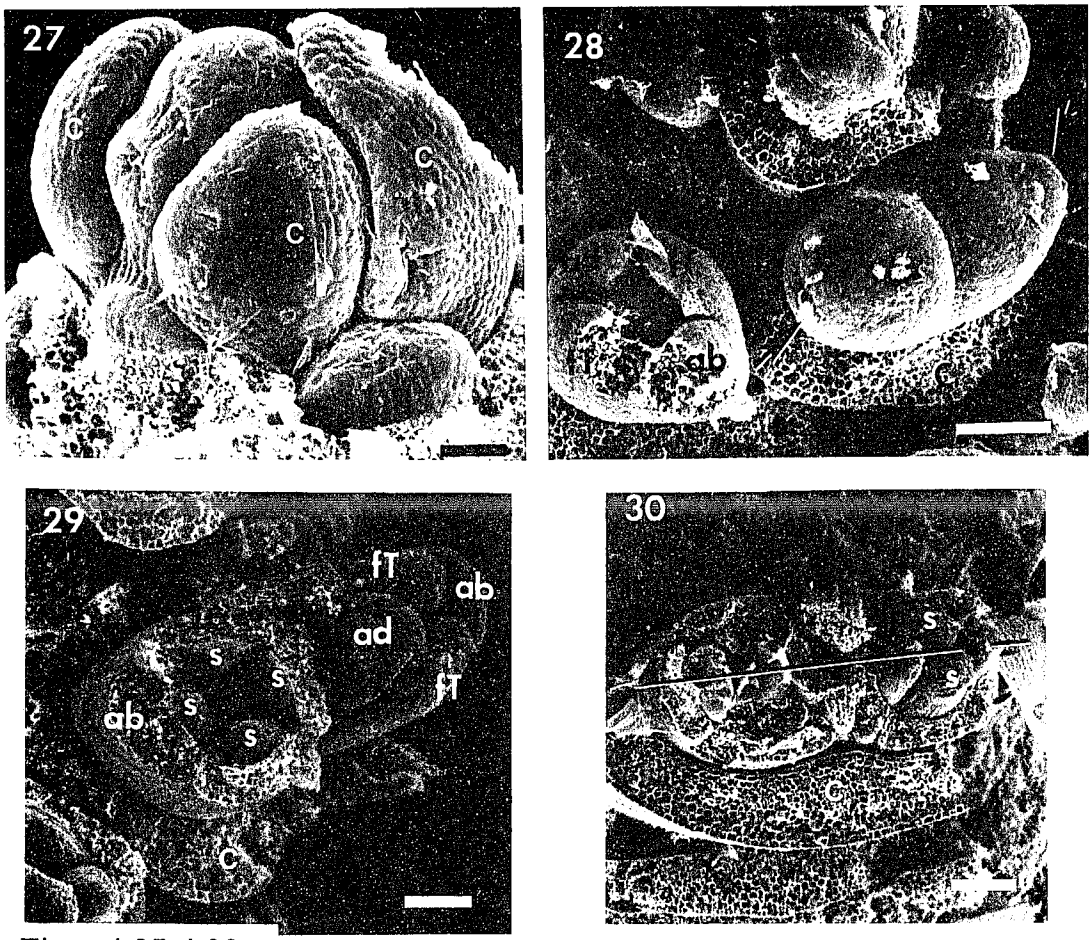
tepal in the adaxial position (example of the right flower in Fig. 4.24). The tepal in the abaxial position is initiated last (Fig. 4.24). In some flowers, the laterally positioned tepals appear simultaneously, prior to the simultaneous initiation of the sagittal pair (Fig. 4.22). After enlargement and aestivation of the tepals, the frontal tepal tips converge and come into contact. The sagittal tepal tips occupy the spaces between the frontal tepals producing sagittally-incomplete aestivation (Fig. 4.25). The flowers of a pair are frontally bisymmetrical (Fig. 4.25).

Eucarpha deplanchii - (monotypic; New Caledonia) (Figs. 4.27-30) - From the axils of bracts along a primary inflorescence axis, frontally oblate meristems are initiated (Fig. 4.27), becoming short-shoot meristems. In lateral positions on each side of the bisymmetrical short-shoot meristem, a bract is initiated. A floral meristem then enlarges in the axil of each floral bract (Fig. 4.28). The lateral tepals are initiated first, followed in close succession by the tepals in the sagittal plane (not illustrated). At aestivation the tepals are sagittally incomplete valvate (Fig. 4.29). The flowers of a pair remain frontally bisymmetrical (Fig. 4.30).

Flowers of Knightia excelsa (monotypic; New Zealand), have the same sagittally incomplete valvate tepals at aestivation. The flowers of a pair are frontally bisymmetrical.

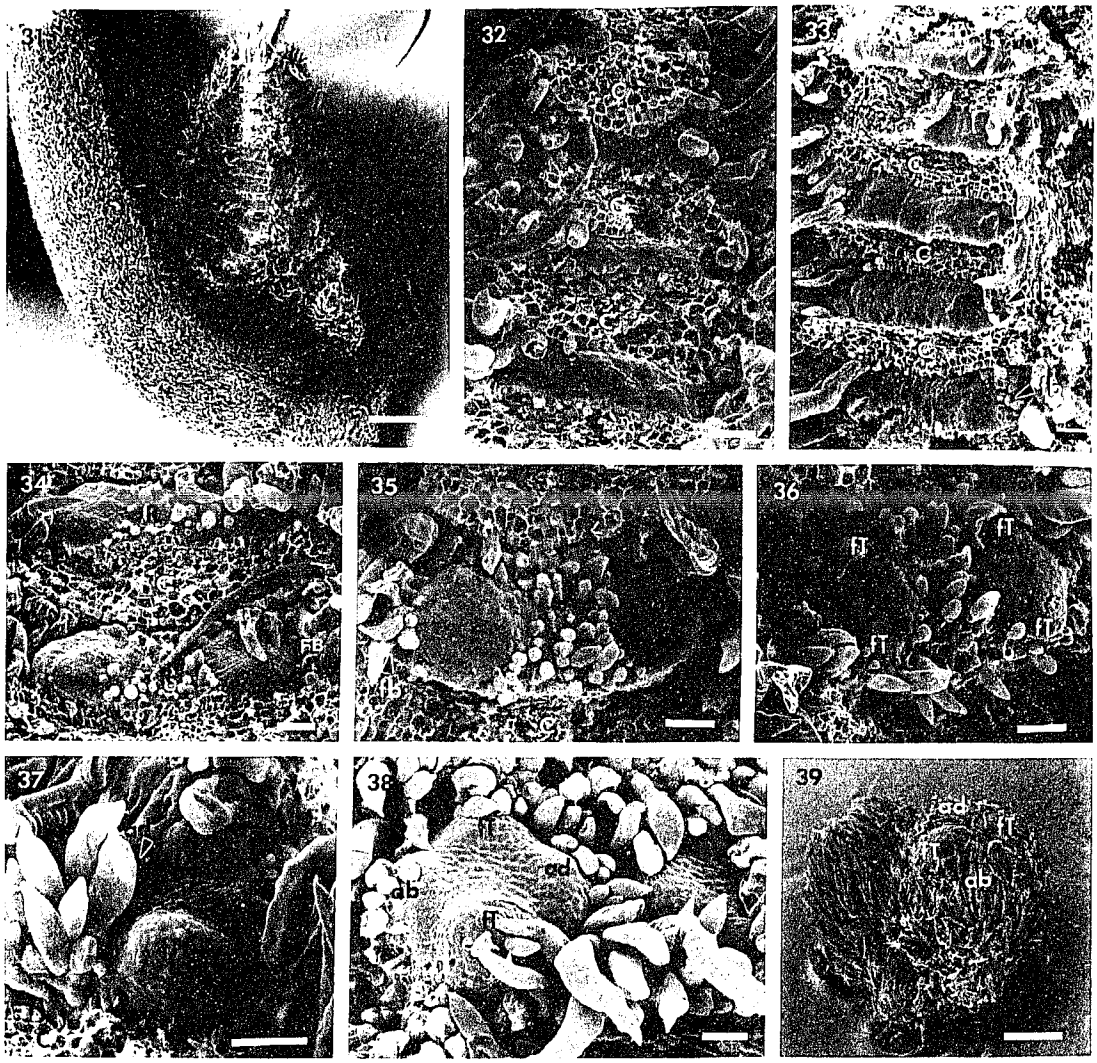
Cardwelliinae - Cardwellia sublimis is a monotypic species from northeast Queensland. (Figs. 4.31-39)--The inflorescence is terminal (auxotelic) and heterothetic bearing secondary (Fig. 4.31) and sometimes tertiary uniflorescence axes. Common bracts are initiated in a tetrastichous phyllotaxy (Fig. 4.31). A frontally oblate meristem is initiated in the axil of common bracts (Fig. 4.32) and becomes the short-shoot meristem. The short-shoot meristem broadens and from each side, a slight protuberance or a bract primordium becomes evident, on which there is precocious differentiation of trichomes (arrowheads in Fig. 4.33). The individual floral bracts will enlarge slightly as will the

Figs. 4.27-30. Flower pair initiation and pattern of aestivation in Eucarpha deplanchii. **27.** Lateral view of young inflorescence apex showing acropetal initiation of common bracts and the development of short-shoot meristems in each common bract axil. **28.** Oblique view of floral meristem initiation showing the two floral bract primordia (arrows) subtending each enlarging floral meristem. **29.** Oblique abaxial view of left flower with the tepals removed showing the superpositioned stamen primordia. The right flower shows the sagittally incomplete pattern of aestivation. **30.** Polar view of flower pair in common bract axil. The pair of flowers are bisymmetrical. **Scale bars**: 27 = 50μm; 28-30 = 100μm.



Figs. 4.27-4.30

Figs. 4.31-39. Flower pair initiation and floral organogenesis in Cardwellia sublimis. **31.** A young secondary inflorescence axis removed from the primary, heterothetic compound raceme. Numerous common bracts are present in four tetrastichous rows, of which only three are visible. **32.** Short-shoot meristems in the axils of common bracts (removed). The short-shoot meristems enlarge acropetally, the lower ones in the image being larger than the upper ones. **33.** A later stage of short-shoot meristem enlargement. Five sequential stages of short-shoot meristems are present. The short-shoot meristem enlarges and is bisymmetrical at the time of floral bract initiation (arrowheads). **34.** Short-shoot as the floral meristems are initiated. Trichomes develop on the short-shoot apical residuum. **35.** Polar view of flower pair showing the enlargement of the floral meristems in the axils of reduced floral bracts. Trichomes have differentiated on the apical residuum of the short-shoot and on the floral bracts (arrowheads). **36.** Slightly oblique polar view of flower pair. The first two tepals have been initiated; each one in a lateral position along the frontal plane. **37.** Right flower of a pair showing the initiation of the adaxial (arrow) and abaxial tepals. **38.** Left flower of a pair showing an apparent simultaneous initiation of all four tepals. **39.** Flower pair removed from principal axis showing the sagittally incomplete valvate aestivation pattern. **Scale bars**: 31=500μm; 32-38=50μm; 39=250μm.



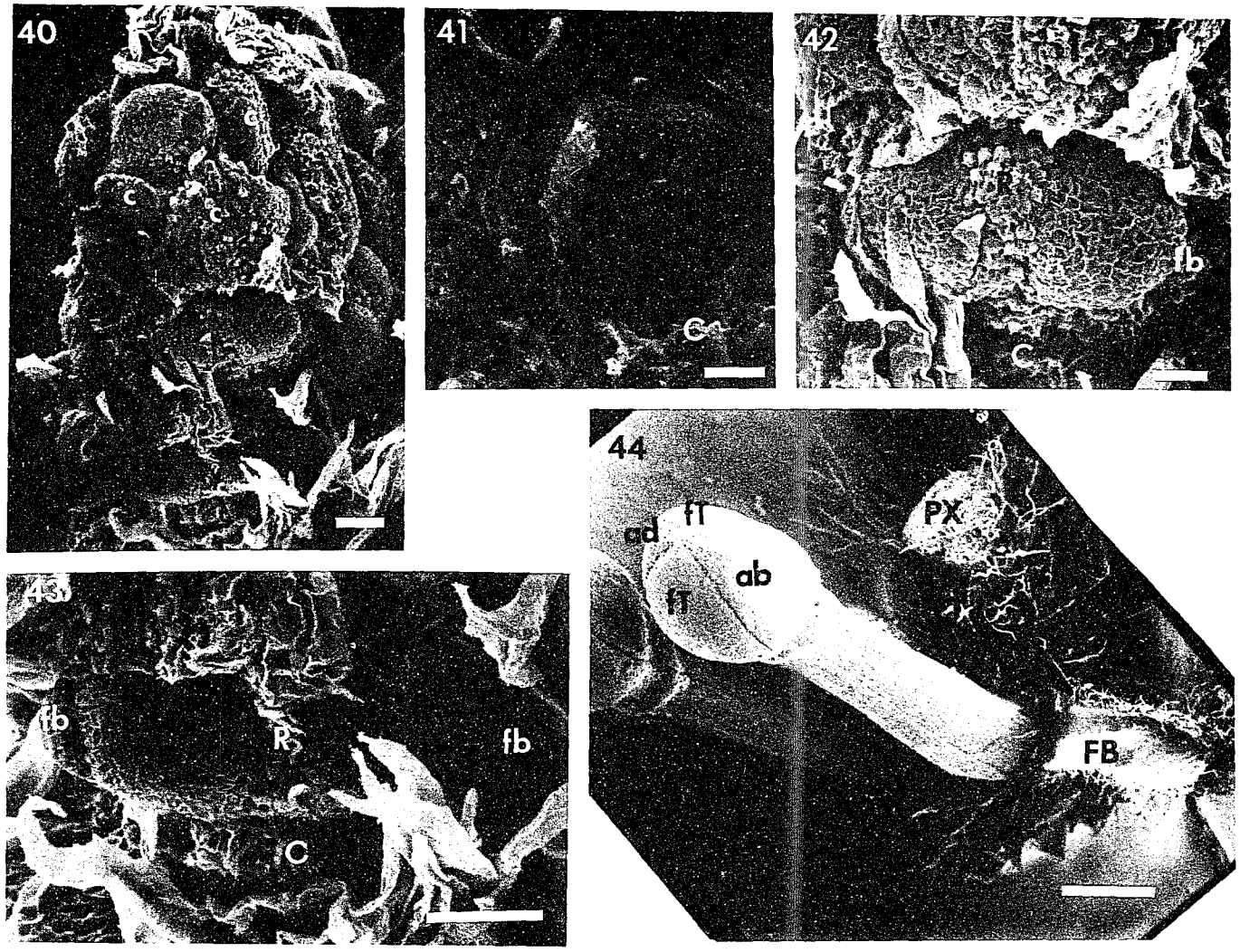
Figs. 4.31-4.39

trichomes (arrowhead in Figs. 4.34, 35). Two floral meristems are initiated, one on each side of the short-shoot, in the axil of each floral bract (Figs. 4.34, 35). Between the floral meristems, trichomes differentiate on the unused portion of the short-shoot meristem residuum (Figs. 4.34-36). The tepals appear to be initiated in a simultaneous whorl (Fig. 4.36, 38). On the lower portions of the principal inflorescence axis, the tepals are initiated sequentially; the lateral tepals are initiated before the sagittal tepals and appear larger (Fig. 4.37). Tepal aestivation varies in flowers of an inflorescence in this taxon. The typical sagittally-incomplete valvate pattern of aestivation occurs in 69% of the flowers (Fig. 4.39). The completely valvate aestivation pattern, in which the four tepals come into contact with one another occurs in 26% of the flowers. The other 5% of the flowers are frontally-incomplete valvate, in which the sagittal tepal tips come into contact with one another, the lateral tepal tips not touching one another (200 flowers randomly sampled on 25 inflorescences from two different collections). The flower pairs are frontally bisymmetrical.

Oriteae - This tribe is made up of two genera, Orites and Neorites. Only partial stages of developmental material of Orites revoluta of Tasmania were available and mature stages of Orites excelsa and Neorites kevediana. Orites revoluta is described.

Orites revoluta - (Figs. 4.40-44) - The primary shoot is auxotelic, the vegetative shoot terminating with a simple raceme or spike (Fig. 4.40). Flowers have short pedicels. Within the axil of each common bract, a frontally oblate short-shoot meristem is initiated (Figs. 4.40-41). From the sides of the short-shoot meristem, two bract primordia are initiated (Figs. 4.42-43). A floral meristem develops proximal to each bract and the floral bracts enlarge (Fig. 4.43). Trichomes differentiate on the apical residuum between the two flowers (Fig. 4.42). In some cases, the floral bracts will continue to enlarge (Fig.44) but usually they are suppressed ab initio. The frontal tepal tips come into contact with one

Figs. 4.40-44. Short-shoot initiation and aestivation pattern in Orites revoluta. **40.** Young inflorescence showing the arrangement of common bracts and the development of short-shoot meristems in each common bract axil. **41.** Short-shoot meristem. **42.** Polar view of short-shoot. Floral bracts have been initiated, one on each side of the short-shoot, and a floral meristem is enlarging in each floral bract axil. Trichomes are beginning to differentiate on the residuum of the short-shoot. **43.** Polar view of flower pair showing the enlarged floral meristems in floral bract axils. The flowers of a pair are arranged bisymmetrically. **44.** Pre-anthesis buds showing the sagittally incomplete valvate pattern of aestivation in the right flower and the anomalous enlarged floral bract. **Scale bars**: 40 and 43=50μm; 41-42=25μm; 44=500μm.



Figs. 4.40-4.44

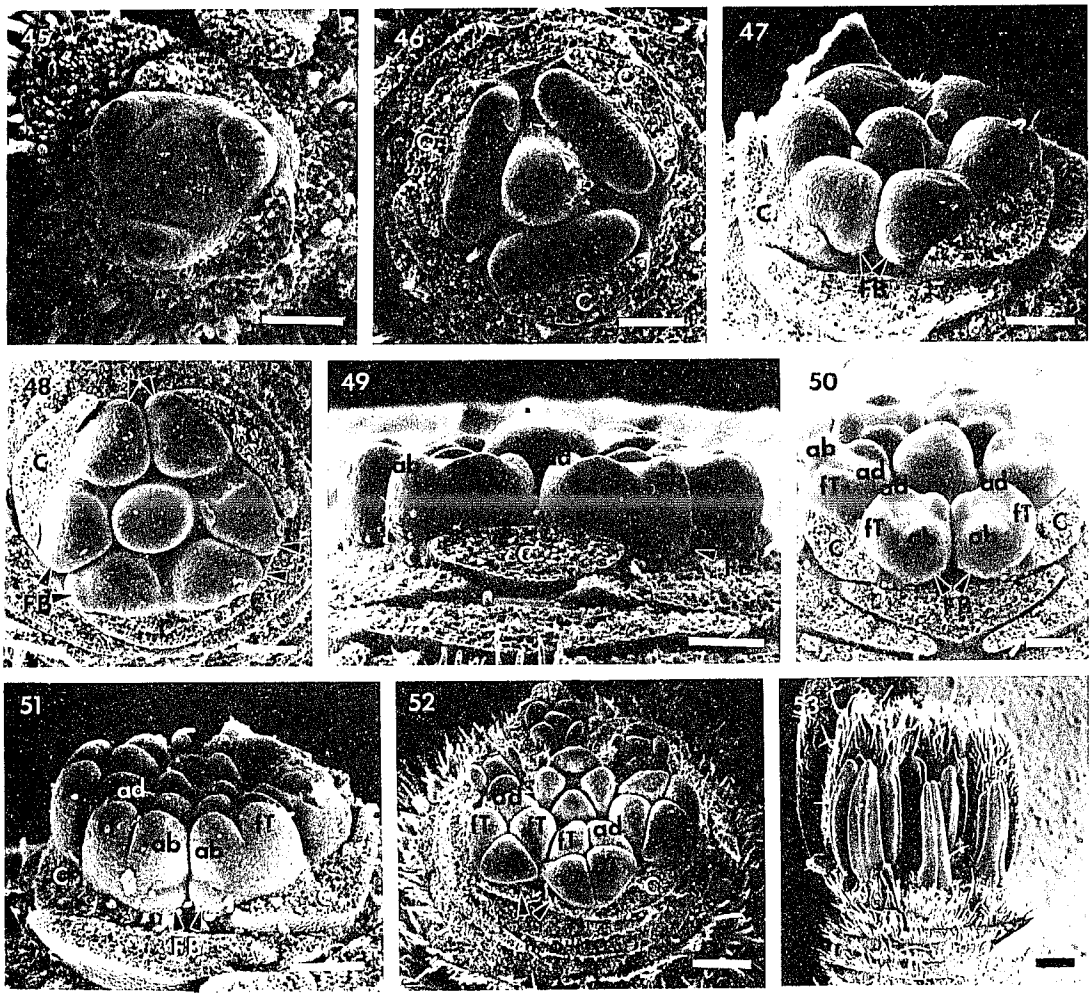
another and the sagittal tepals tips do not touch one another thus are sagittally-incomplete valvate at aestivation (Fig. 4.44). The flower pairs do not have an elongate peduncle. The flower pairs are frontally bisymmetrical.

Inflorescences of Orites excelsa and Neorites kevediana develop in the axils of foliage leaves (Venkata Rao, 1971, Wrigley and Fagg, 1988). Mature flowers (not illustrated) of these species lack individual floral bracts. The tepals are sagittally-incomplete valvate at aestivation and the flower pairs are frontally bisymmetrical.

Macadamieae - Macadamieae (sensu Johnson and Briggs, 1975) includes six subtribes. In the limited developmental material available, tepal aestivation in all investigated taxa is sagittally incomplete valvate.

Lambertiinae - Lambertia inermis - (Figs. 4.45-53) - The inflorescence is terminal (Fig. 4.53). The whorled leaf primordia become serially transformed into cuneate bracts (bases in Fig. 4.45) in the capitate, seven (6-8)-flowered inflorescence. A frontally broad, bisymmetrical short-shoot meristem (Fig. 4.46) develops in the axils of each of the last three bracts initiated (Fig. 4.45). The remaining apical meristem of the principal axis remains undifferentiated and domelike (Fig. 4.46). Six floral meristems are initiated: one on each side in the frontal plane on each of the three short-shoot meristems (Fig. 4.47-48). Prior to and during the enlargement of the floral meristems, an individual floral bract initiates on each side on the short-shoot meristem flanks (arrowheads in Figs. 4.47-48), subtending each floral meristem. The floral bracts are then suppressed and do not enlarge further (arrow in Fig. 4.52). Trichomes differentiate on the floral bract (arrowheads in Figs. 4.49-51). The flowers in a pair become slightly askew, deflected abaxially in relation to the principal axis, as do the subtending floral bracts as development continues (compare Figs. 4.48, 50, 52). The tepals are initiated in an apparently rapid, almost simultaneous succession (Fig. 4.49) although the frontal tepals appear slightly

Figs. 4.45-53. Flower pair development and floral organogenesis in Lambertia inermis. The inflorescence is oriented so that a common bract is positioned in the lower part of each image except for 47, 50 and 51. **Fig 45**. Polar view of the young capitate inflorescence. Three common bract primordia are present. **46**. Polar view of young inflorescence showing the bisymmetrical enlargement of the short-shoot meristems. The principal inflorescence apex (PX) is actinomorphic. **47**. Oblique view of inflorescence showing the initiation of floral bracts (arrow heads), one on each lateral side of the short-shoot meristem. Each floral bract subtends a floral meristem. A floral bract primordium is initiated from the principal axis residuum (arrow). **48.** Polar view of similar stage showing the arrangement of the floral pair meristems. **49**. Abaxial view of flower pair showing the apparent simultaneous initiation of the tepals although the abaxial tepals tend to be smaller. **50**. Oblique view of inflorescence showing the dorsiventral arrangement of the tepals relative to the individual floral bracts. **51**. Oblique view of inflorescence and flower pairs showing the cessation of development of the floral bracts and the differentiation of trichomes. A single flower has developed from the principal axis residuum (arrow head) and is developmentally behind the encircling flowers. **52.** Polar view of inflorescence at aestivation showing the sagittally incomplete valvate pattern of aestivation. The flowers of a pair have become concavely skewed. **53**. Side view of older inflorescence showing the capitate form. **Scale bars**: 45-50 and 52=100 μm; 51 and 53=250 μm.



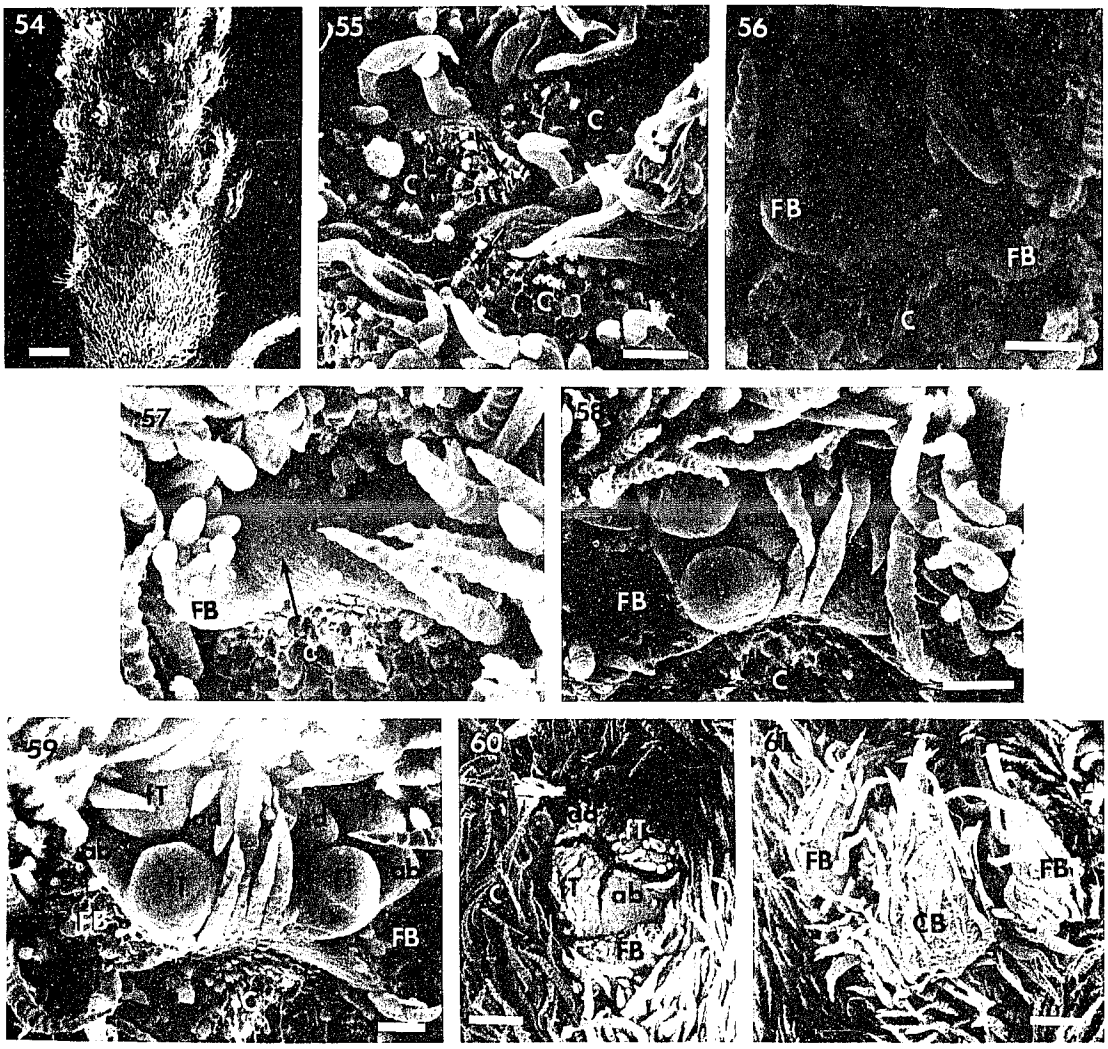
Figs. 4.45-4.53

larger than the sagittal tepals (Fig.51). The tepals are sagittally-incomplete valvate at aestivation, the same as in other proteaceous taxa (Fig. 4.52). As the inflorescence broadens through developmental time, the flower pairs become concavely-askew in relation to the frontal plane (Figs. 4.3B; 52).

The principal inflorescence apex often develops into a single flower (Fig. 4.52). Although it appears to be terminal, a small bract primordium is initiated (arrow in 47). Aestivation of the central flower is sagittally incomplete valvate. Other species in the genus have been described as having even numbers of flowers per inflorescence (Venkata Rao, 1968) or even a single flower. A more thorough developmental investigation of other taxa in the genus is necessary, prior to any conclusions about the report of a single flower.

Hicksbeachiinae - Virotia deplanchii (New Caledonia; Figs. 4.54-61) - Numerous flowers develop along the axillary racemes (Fig. 4.54). A frontally oblate short-shoot meristem develops within the axil of each common bract (arrow, Fig. 4.55). The short-shoot meristem becomes slightly concavely arcuate, deflected abaxially in relation to the frontal plane. A floral bract primordium is initiated on each side of the short-shoot meristem, each positioned slightly abaxially from the median frontal plane (Fig. 4.56). The floral bracts continue to develop and trichomes differentiate on the tips (Fig. 4.57). A floral meristem initiates and enlarges within the axil of each bract (Fig. 4.57). Trichomes differentiate on the apical residuum of the short-shoot meristem (Fig. 4.57). The frontal tepals appear to be initiated first (they are larger in Fig. 4.58), followed by the tepal in the adaxial position, and lastly the tepal in the abaxial position (Fig.58-59). At aestivation, the tepal arrangement is typical, sagittally incomplete valvate (Fig.60). The flower pairs are concavely askew (Fig. 4.3C) in relation to the frontal plane of the pairs (Fig. 4.3B). The floral bracts are well developed (Fig. 4.61).

Figs. 4.54-61. Flower pair development and floral organogenesis in Virotia leptophylla. **54**. Lower half of young inflorescence with numerous common bracts, some of which have been removed. **55**. Short-shoot meristems in the axils of the common bracts. **56**. Two floral bracts have been initiated on the short-shoot meristem, one on each side although positionally they are concavely skewed. **57.** A floral meristem is initiated in the axil of each floral bract. Trichomes differentiate on the floral bracts and on the short-shoot residuum. **58**. Three of four tepals are present on the left flower of the pair. The lateral tepals are larger than the adaxial tepal and there is no tepal in the abaxial position. **59**. The abaxial tepal has been initiated in both flowers of a pair. **60**. Abaxial view of right flower of a pair showing the sagittally incomplete valvate pattern of aestivation; the floral bract has been removed and the common bract is to the left in the image. **61**. Polar view of short-shoot; the common bract and the floral bracts have not been removed. **Scale bars**: 54=500μm; 55-59=50μm; 60-61=100μm.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Figs. 4.54-4.61

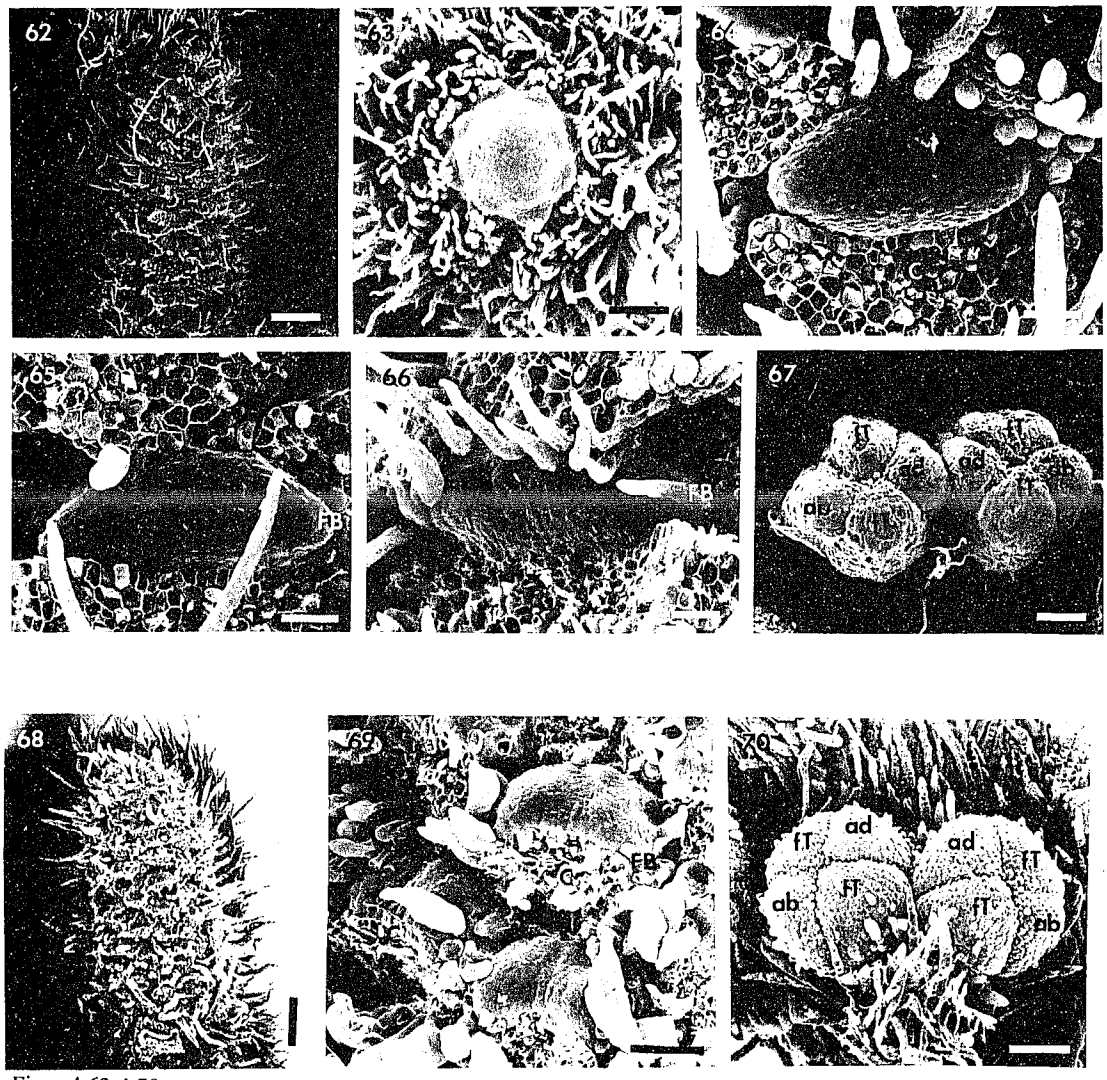
Flowers of Athertonia diversifolia had the same sagittally-incomplete aestivation pattern, the same concavely-askew flower pairs and relatively well developed floral bracts.

Macadamiinae - (three genera) (Figs. 4.62-70). Partial development stages of two genera were available.Braebium stellatifolium - (African, monotypic: Figs. 4.62-67) - From the axils of leaves, simple racemes develop (Fig. 4.62). Common bracts are initiated acropetally (Figs. 4.62, 63) by the inflorescence apex. Within the axil of each common bract, a bisymmetrical short-shoot meristem is initiated (Fig. 4.64). The short-shoot meristem enlarges and becomes slightly concavely arcuate in relation to the frontal plane (Fig. 4.65). A floral bract develops (FB, Fig. 4.66) on each side of the slightly arcuate short-shoot meristem. Tepal organogenesis was not observed although, at aestivation, the tepals are sagittally incomplete valvate. The flower pairs are slightly concavely askew in relation to the frontal plane of the pairs (Figs. 4.3B, 67).

Macadamia integrifolia (Figs. 4.68-70) - From the axils of leaves or leaf-scars, simple racemes form that resemble bottle-brushes. Common bracts are initiated acropetally by the inflorescence apex along the principal inflorescence axis (Fig. 4.68). A short-shoot meristem is initiated within the axils of each common bract (Figs.68, 69). The frontally oblate short-shoot meristem enlarges and becomes slightly concavely arcuate in relation to the frontal plane (lower primordia in Fig. 4.69). Although no floral bracts are present, trichomes differentiate in the positions where floral bract primordia would be expected (not illustrated). Tepal initiation was not observed due to a lack of material. At aestivation, the tepals are sagittally-incomplete valvate (Fig. 4.70). The flower pairs are concavely skewed (Fig. 4.3B) in relation to the frontal plane of the pairs.

Helicieae - (no illustrations) - Helicieae is composed of three subtribes, Hollandaeinae (monotypic), Heliciinae (Helicia and Xylomelum), and Triuniinae (monotypic). Early ontogenetic stages of these taxa were not available.Aestivation

Figs. 4.62-70. Short-shoot meristem initiation and aestivation patterns in Macadamiinae. Figs. 4.62-67. Braebium stellatifolium. **62.** Young inflorescence, the numerous common bracts having been removed. **63.** Polar view of inflorescence apex showing the initiation of the common bracts. **64.** Polar view of short-shoot meristem in common bract axil. The meristem is bisymmetrical. **65.** Polar view of older short-shoot meristem showing the differential enlargement as floral bracts are initiated on each side. The short-shoot becomes slightly concavely skewed. **66.** Oblique lateral view of short-shoot showing the initiation of the floral bracts and the development of trichomes on the floral bracts. **67.** Polar view of flower pair showing the pattern of sagittally incomplete aestivation. **Scale bars**: 62=250μm; 63 and 67=100μm; 64-66=25μm. **Figs. 4.68-70.** Macadamia integrifolia. **68.** Young inflorescence with numerous common bracts, many of which have been removed. **69.** Short-shoot meristems in common bract (removed) axils. The meristems are slightly concavely skewed. Small floral bracts appear to be initiated on the lateral flanks of each short-shoot meristem. **70.** Polar view of flower pair showing sagittally incomplete valvate aestivation and the concavely skewed flower pair. **Scale bars**: 68=250μm; 69=50μm; 70=100μm.



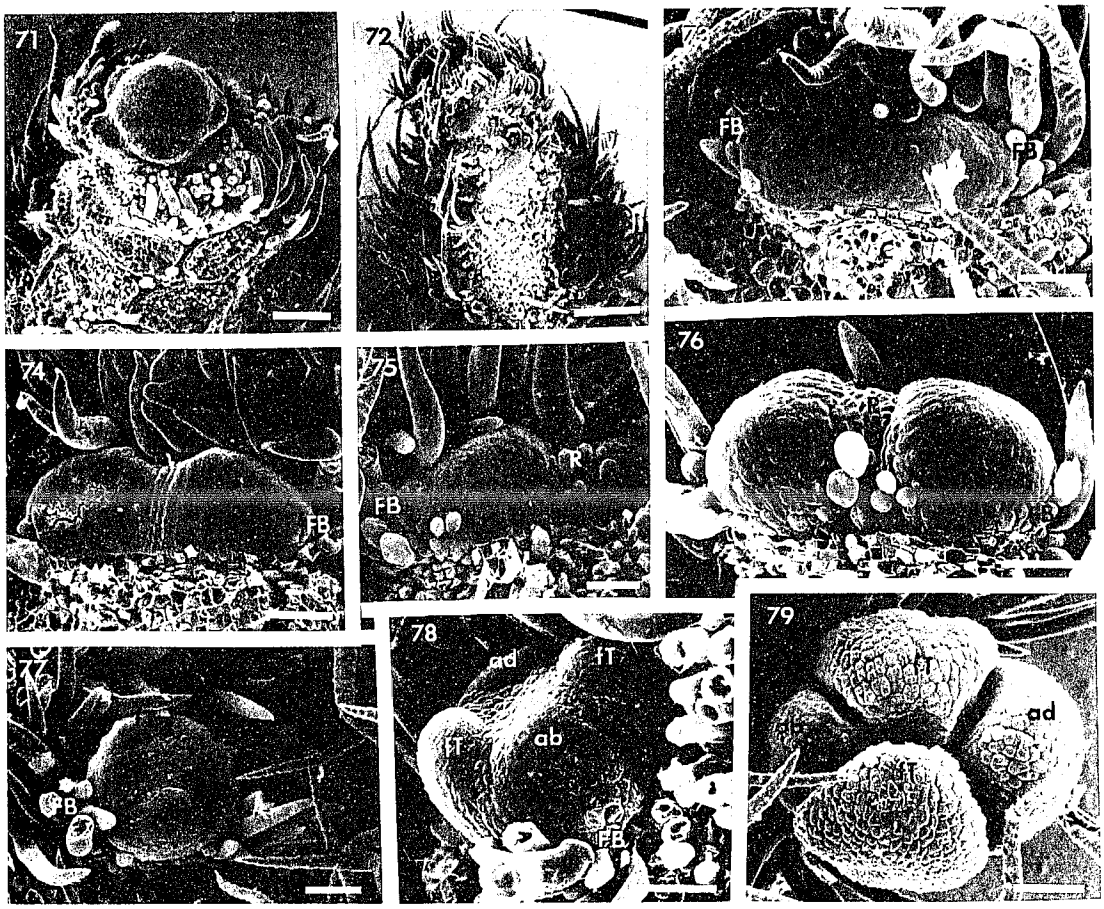
Figs. 4.62-4.70

patterns are the same in all taxa, sagittally incomplete valvate (Fig. 4.3D). Hollandaea sayerana and Helicia species have relatively well developed floral bracts and the flowers of a pair are concavely skewed (Fig. 4.3B) in relation to the frontal plane of the flower pairs.

Embothrieae - (Figs. 4.71-112) - Embothrieae is composed of four subtribes: Buckinghamiinae, Stenocarpiinae, Lomatiinae and Embothriinae. Ontogeny will be described for one representative species in each.

Buckinghamiinae - (two genera) - Opisthiolepis heterophylla - (Figs. 4.71-79) - The plants of the monotypic O. heterophylla have what appear to be axillary inflorescences. Developmental studies indicate that the single racemose axis is the terminus of the primary shoot axis, so the growth of the plant is sympodial. Common bracts are initiated acropetally along the inflorescence apex (Fig. 4.71). Within the axil of each common bract a bisymmetrical meristem is initiated (Fig. 4.72). On each side of the short-shoot meristem, in the median frontal plane, an individual floral bract is initiated (arrows in Figs. 4.73-75). A floral meristem is initiated within the axil of each floral bract (Figs. 4.74-75). Trichomes form on each floral bract (Fig. 4.75-76) and the bracts do not enlarge (Fig. 4.78). Trichomes are produced on the apical residuum, between the enlarging floral meristems (Fig. 4.75-76). The tepals in lateral positions (in the median frontal plane) are initiated first (Fig. 4.77) followed by the initiation of the adaxial and abaxial sagittal tepals (Fig. 4.78). After aestivation, the tepals are sagittally-incomplete valvate (left flower of pair shown in Fig.79). The flower pairs are bisymmetrical (Fig. 4.3A). Each flower of a pair will rotate: the left flower rotates counter-clockwise and the right flowers rotate clockwise as a result of pedicel elongation and twisting (not illustrated).

Figs. 4.71-79. Flower pair development and floral organogenesis in Opisthiolepis heterophylla. **71**. Oblique polar view of young inflorescence showing the inflorescence apex and initiation of common bract primordia. **72**. Side view of older inflorescence with some of the common bracts removed. **73**. Short-shoot meristem in the axil of a common bract. Floral bracts have been initiated, one on each side of the short-shoot meristem. **74**. Initiation of floral meristems. **75**. Left floral meristem on two-flowered short-shoot showing the initiation of trichomes on the floral bract and on the short-shoot residuum. **76.** Abaxial view of flower pair showing the enlargement of each of the two floral meristems and the suppression of floral bracts. **77**. Polar view of left flower of pair showing the initiation of the first two tepals in the frontal plane of the flower. **78**. Abaxial sagittal view of right flower of floral pair showing the vestiges of the floral bract and the initiation of the adaxial and abaxial tepals. **79**. Polar view of left flower of a pair showing the sagittally incomplete pattern of aestivation. **Scale bars**: 71=100μm; 72=250μm; 73-79=50μm.



Figs. 4.71-4.79

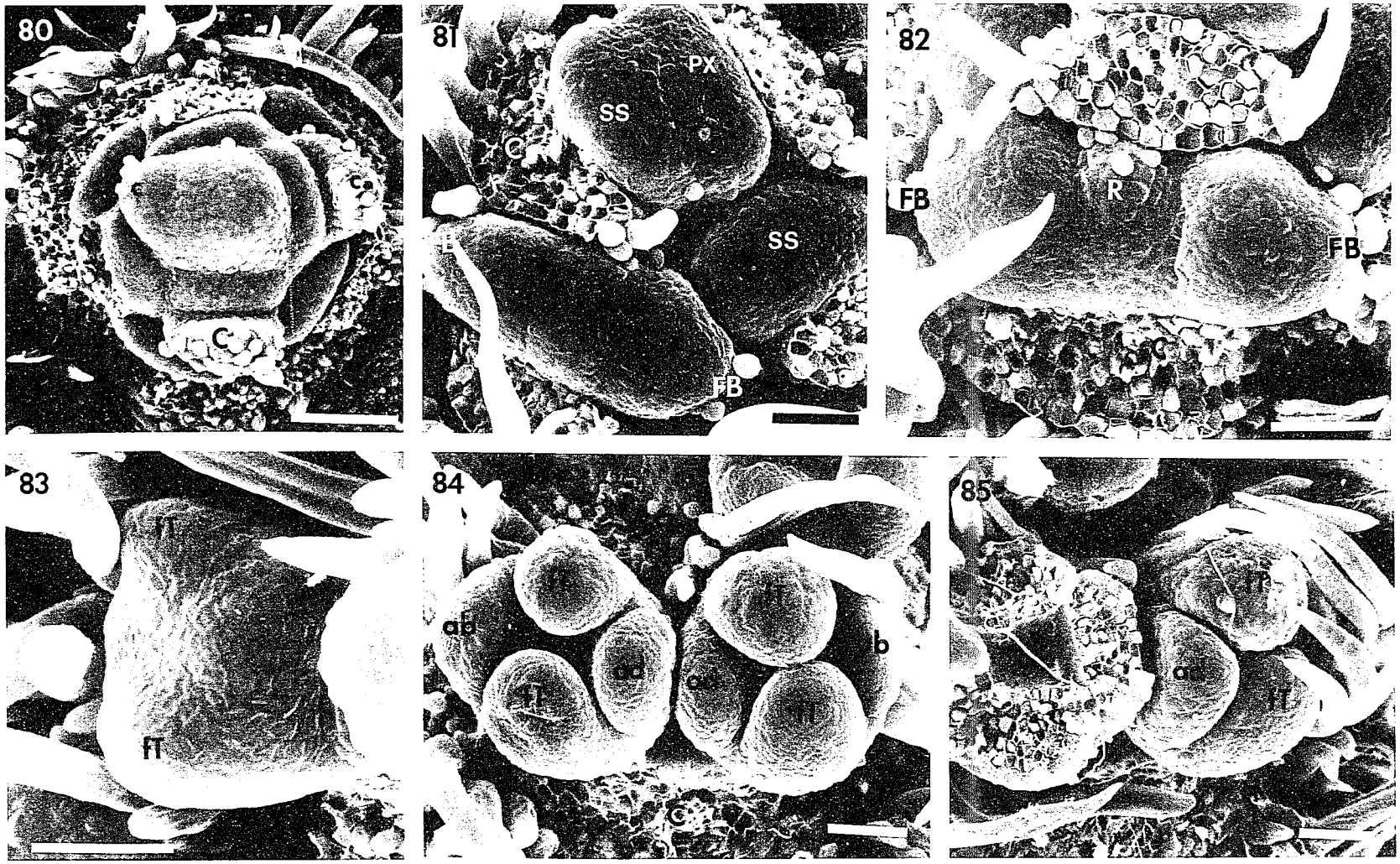
In Buckinghamia celsissima flower pair development and floral ontogenies as well as aestivation patterns and flower pair symmetry are similar to those of O. heterophylla.

Stenocarpiinae - (two genera) - Stenocarpus salignus R. Br.- (27 spp. in Stenocarpus) - (Figs. 4.80-85) - The 18-30 creamy white flowers are arranged in a condensed inflorescence or umbel in a leaf axil towards the end of a branch. Common bracts are initiated acropetally on the principal inflorescence apex (Fig. 4.80). A bisymmetrical short-shoot meristem is initiated within the axil of each common bract (Fig. 4.80-81). A single floral meristem is initiated on each side of the short-shoot meristem in the frontal plane (Fig. 4.81-82). Individual floral bract primordia are initiated concomitant with the initiation of the floral meristems, although the floral bracts are suppressed from further development (arrows in Fig.82). The floral bracts are usually visible towards the base of each inflorescence. The two lateral tepals in the frontal plane are initiated first (Fig. 4.83) followed by the relatively rapid initiation of the tepals in the sagittal positions (Fig. 4.84). After aestivation, the tepals are sagittally-incomplete valvate (Fig. 4.85). The flower pairs are bisymmetrical. At later stages, the pedicels twist, sot that the left flowers rotate counter-clockwise and the right flowers rotate clockwise. Similar development was found in Stenocarpus davallioides. Material of the other genus, Strangea (3 spp.), was not available for study.

Lomatiinae - (one genus:12 species) - Lomatia fraxinifolia - (Figs. 4.86-100) - Heterothetic racemose inflorescences are produced at the ends of branches (auxotelic-Fig. 4.86). A young secondary uniflorescence axis is marked by the arrow in Fig. 4.86. The number of flowers per axis is approximately 16-28.

Most of the nodes of an inflorescence axis have a common bract and two flowers from a short-shoot meristem (Figs. 4.86-94). Common-bract primordia are initiated acropetally by the inflorescence apex (Fig. 4.87). Frontally oblate, bisymmetrical

Figs. 4.80-85. Floral pair development and floral organogenesis in Stenocarpus salignus. **80.** Polar view of young inflorescence. The common bract primordia are initiated in a tetrastichous pattern, and short-shoot meristems (**SS**) are initiated in each common bract axil. **81.** Oblique view of sequential developmental stages of short-shoot meristem. At the top, a bilaterally symmetrical short-shoot meristem is initiated. At a later level, in the middle of the image towards the right side, a short-shoot meristem has enlarged. Floral bracts are initiated, one from each side of the short-shoot meristem at the bottom left. **82.** Polar view of short-shoot illustrating floral bracts and the floral meristems in each floral bract axil. The short-shoot apical residuum elongates slightly. **83.** Adaxial view of left flower of pair; the floral bract is behind the flower. The two lateral tepal primordia have been initiated. **84** Polar view of floral pair showing the initial contact between the two lateral tepals. **85.** Polar view of floral pair. The tepals have been removed in the left flower, and the right flower shows the sagittally incomplete valvate pattern of aestivation. **Scale bars**: 80, 82 = 100μm; 81, 83-85 = 50μm.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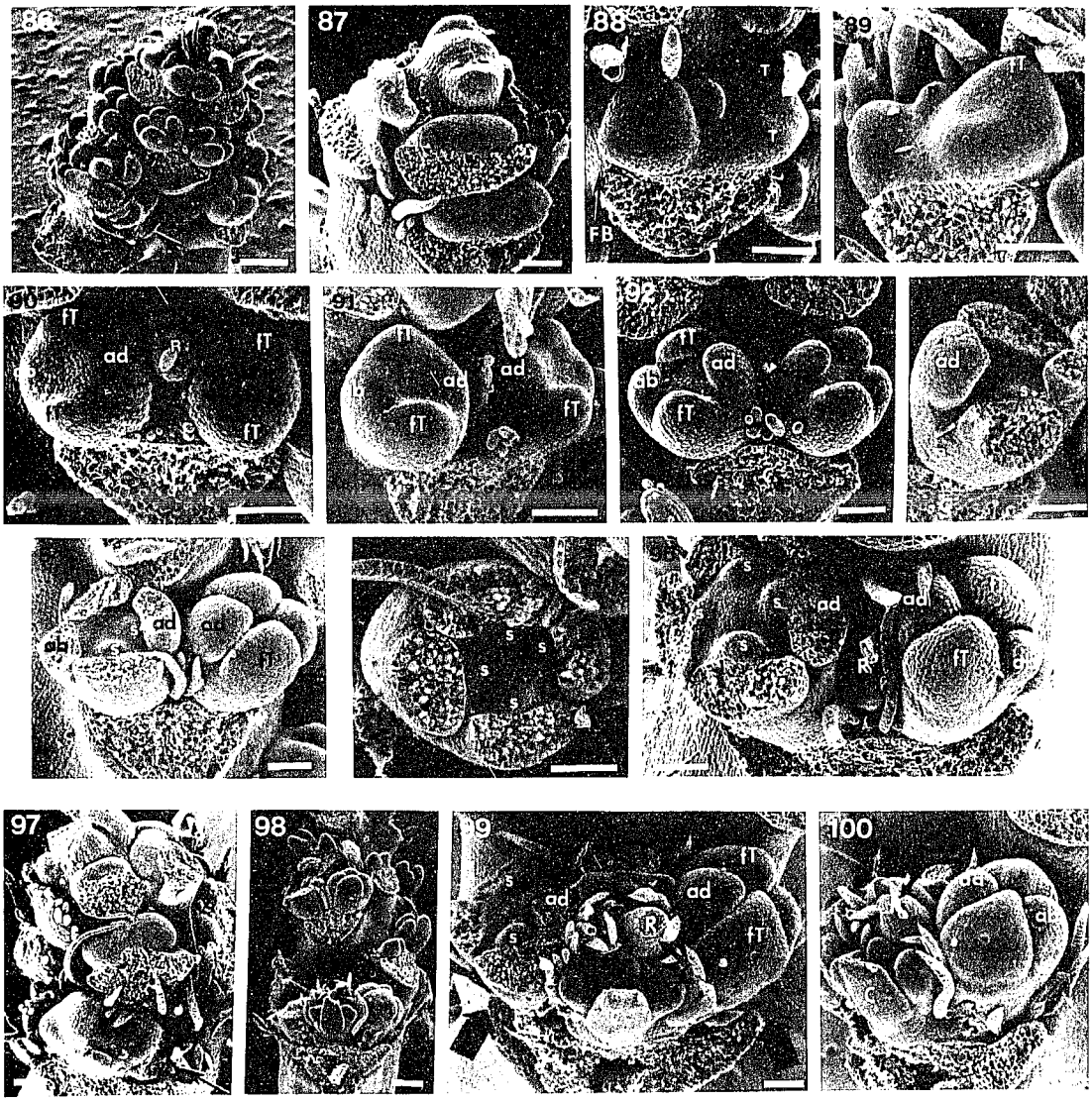
Figs. 4.80-4.85

meristems develop wthin the axil of each common bract (Fig. 4.87). A floral meristem is initiated on each side of the short-shoot meristem in the frontal plane (Fig. 4.88). In some cases, what appear to be individual floral bracts are initiated on the lateral flanks, distal to each floral meristem (FB, Fig. 4.88). The floral bracts are suppressed from further development (Fig. 4.89). Between the floral meristems, the apical residuum expands for a short duration (R, Figs. 4.88-90), becoming a pronounced protuberance in some cases (arrow in Fig. 4.92) . The first tepals initiated are in the frontal plane of each flower (right flower in Figs. 4.89, 90), followed by the sagittal tepals (left flower in Fig. 4.90 and Figs. 4.91, 92). The stamen primordia are initiated in the same sequence as the tepals, and in positions superposed or opposite each tepal; the first stamen primordium is initiated in the frontal plane closest to the common bract (Fig. 4.93) followed by the stamen in the opposite frontal position and that in an adaxial position (Fig. 4.94-95). The last stamen primordium initiated is in the abaxial position (Fig. 4.96). Tepal aestivation is by lateral tepal tip convergence (slightly separated in right flower in Fig. 4.96). The flower pairs are bisymmetrical until tepal aestivation (Fig. 4.94). After aestivation the flowers in a pair rotate away from one another, the left flower counter-clockwise, the right flower clockwise due to twisting and expansion of the principal axis and pedicels(Fig. 4.96).

Developmental and morphological variation is present in the development of secondary uniflorescence axes in the material examined. In most material, the secondary uniflorescence axes produce common bracts and flower pairs (arrows in Fig. 4.86). In some material, the basal-most common bracts are suppressed and a single flower develops on each side (Figs. 4.97-100). Between the two flowers, the short-shoot apex continues growth, the secondary uniflorescence axis elongates and common bracts are initiated (Fig. 4.97, 99). Along the secondary axis, above the two basal most nodes, in the axil of each common bract, a short-shoot meristem is initiated and a pair of flowers will develop (Fig. 4.99). In the two basal most nodes, in the axils of suppressed bracts, a floral meristem is

Figs. 4.86-100. Floral pair development and floral organogenesis in Lomatia fraxinifolia. **86.** Side view of young compound inflorescence. At lower node, on left in image (arrow), a secondary inflorescence axis has been initiated and produces two-flowered short-shoots at each subsequent node. **87.** Side view of young inflorescence showing the initiation of common bracts and short-shoot meristems in each common bract axil. **88.** Polar view of short-shoot. Two floral meristems have been initiated and a floral bract is present in the left flower. The two lateral tepals have been initiated on the right flower (arrows). **89.** Oblique basal view of short-shoot. The frontal tepals have been initiated. **90.** Polar view of short-shoot showing tepal initiation. The frontal tepals are initiated first. **91.** Slightly oblique polar view of left flower of a pair showing the initiation of the adaxial and abaxial tepals. **92.** Oblique polar view of flower pair after the four tepals have been initiated on each flower. The arrangement of the flowers is bilaterally symmetrical. **93.** Right flower of a pair showing the initiation of the first stamen in a lateral position of the frontal plane on the common bract side of the flower. **94.** Polar view of floral pair showing the initiation of the two frontal and the adaxial stamen primordia. The right flower shows the sagittally incomplete pattern of aestivation. **95.** Oblique abaxial view of left flower of a pair showing two stamen primordia in frontal positions. The abaxial side of the flower is to the left in the image. **96.** Older developmental stage of a flower pair showing the pattern of aestivation in the right flower and the presence of all four superposed stamen primordia. The apical residuum of the short-shoot persists (R). **Figs. 4.97-100.** Anomalous secondary inflorescence axis development. **97.** Primary inflorescence axis. At the lowest node (arrow) a secondary inflorescence axis is developing. **98.** Principal inflorescence axis with anomalous secondary inflorescence axis. The secondary axis has a single flower on each side suggesting a proliferation of a short-shoot meristem into a secondary inflorescence axis. **99.** Polar view of anomalous secondary inflorescence axis showing the presence of a single flower on each side at the base of the axis (arrows). Above the two single-flowered nodes, the inflorescence produces common bracts and in each common bract there is a flower pair. **100.** Alternate view of same material showing the pattern of aestivation of the single flower is sagittally incomplete valvate. In addition, there is an apical residuum of the secondary inflorescence (arrow). **Scale bars**: 86, 97=250μm; 87-96, 98-100=100μm.



Figs. 4.86-4.100

initiated (Fig. 4.97). The two single flowers at the base of the uniflorescence have an ontogeny similar to the flowers in pairs described above through aestivation (note lateral stamen primordia present in left flower of 99 and aestivation in right flower of 99). The apical residuum of the secondary inflorescences becomes a spike-like projection (Fig. 4.100), similar to the apical residuum of the blastotelic primary inflorescence axes (not illustrated). It should be noted that the presence of a 'single flower' on each side of the base of secondary uniflorescence axes was observed in only two of 11 inflorescences examined (Fig. 4.98-100). Whether the single-flowered condition is due to secondary axis proliferation (as in some species of legumes with pseudoracemes) or other developmental pressures needs additional investigation. In the other nine inflorescences, each node on the secondary uniflorescences, including the basal two, had two flowers in each common-bract axil (Fig. 4.86).

Embothriinae - (four genera, sensu Crisp and Weston, 1987: Figs. 4.101-112) - The earliest stages of floral development were available only in Telopea mongaensis. It should be noted however that other taxa in Embothriinae differ from T. mongaensis in the following respect. The aestivation patterns of Telopea speciossima (Fig. 4.111), Embothrium coccineum (Fig. 4.112), Alloxylon flammeum and Oreocallis brachycarpa were usually by lateral tepal tip convergence and the flowers in a pair had the concavely-skewed arrangement in relation to the frontal plane of the pairs. In T. mongaensis, the flowers in a pair show the **convexly-skewed** arrangement (Fig. 4.110, 3C). Variation in aestivation patterns of the tepal tips among flowers on the same inflorescence was observed in Embothrium coccineum.

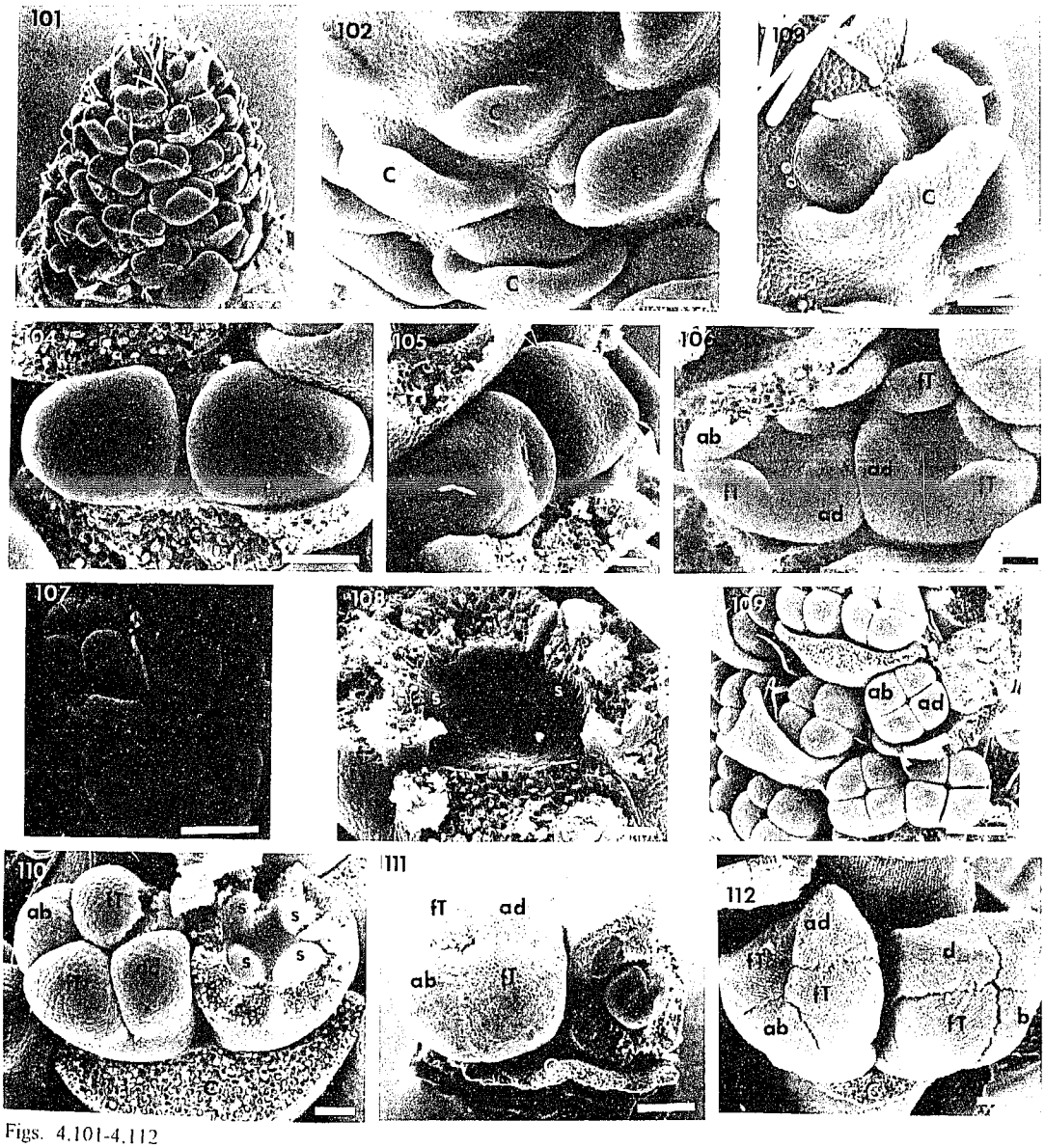
Telopea mongaensis Cheel - (Figs. 4.101-110) - Capitate inflorescences develop at the ends of shoots (auxotelic) and bear numerous highly zygomorphic red flowers. Common bracts are initiated acropetally on the inflorescence apex (Fig. 4.102). Bisymmetrically oblate short-shoot meristems develop within each common bract axil

(Figs. 4.101-102). Floral meristems are initiated on each side of the short-shoot meristem (Fig. 4.103). The floral meristems become distorted as they enlarge and appear to be sagittally oblate (Fig. 4.104). Tepal initiation appears relatively rapid and order is apparently variable, either in pairs or unidirectional. In some flowers, two tepals are initiated simultaneously approximately in the sagittal plane (arrowheads in Fig. 4.105). The same two tepals appear larger at a stage when all four tepals are present (in the right flower of Fig. 4.106). In other flowers (the left flower of Figure 4.106), the largest tepal is on the abaxial side, the frontal tepals are approximately equal in size, and the tepal in the adaxial position is smallest, suggesting a unidirectional order. Generally, no bract primordia are visible due to the deformation of the floral apices from compression (Fig. 4.105-106). Prior to aestivation, the four tepals appear similar in size (Fig. 4.107). Tepal aestivation is sagittally-incomplete valvate; the two tepals in the lateral positions, the ones that were initiated first in most flowers, converge and come into contact with one another over the top of the inner developing floral organs. The sagittal tepal tips do not touch one another (Figs. 4.109-110).

Stamen initiation appears, oddly enough, unidirectional from the abaxial side of the flower. In Figure 4.108, an abaxial view of the right flower, a stamen primordium is present in the abaxial and lateral positions opposite each tepal. Additional materials need to be examined prior to any conclusions on organogeny, particularly when one considers that the convex-askewed arrangement of the flower pairs differs from the other species and genera in the subtribe (Compare the left flowers and the drawn common-line of the pairs in Figures 110-112), although a common line between the flowers and the apical residuum would be convexly-askew in relation to the common bract (Fig. 4.106).

Grevilleae - (Figs. 4.113-133) - Grevillea (~267 spp.) and Hakea (~135 spp.) are both speciose in Australia. Material of the third genus, Finschia (7 spp., New Guinea),

Figs. 4.101-110. Flower pair development and floral organogenesis in Telopea mongaensis. **101**. Young inflorescence showing numerous flower pairs/short-shoots in common bract axils. **102**. Initiation of short-shoot meristems in common bract axils. **103**. Frontally oblique view of the initiation of floral meristems on the short-shoot meristem. **104**. Polar view of short-shoot meristem with two floral-meristems. **105**. Initiation of two frontal tepals (arrows) on floral meristem. **106.** Variation in size and initiation of tepals. The left flower has initiated the four tepals in an apparently unidirectional sequence from the abaxial side. In the right flower, the two frontal tepals are larger and appear to have been initiated prior to the abaxial and adaxial tepals. **107**. Polar view of flower pairs after tepal initiation. The tepals appear to be equal in size. **108**. Abaxial view of left flower in pair, the tepals have been removed to show stamen initiation. The stamen in the abaxial position is initiated first and the stamens opposite the frontal tepals are slightly smaller. **109**. View of side of inflorescence showing the sagittally incomplete valvate pattern of aestivation. **Figs. 4.110-112.** Aestivation of taxa in Embothriinae and the orientation of the flowers in a pair. **110**. Telopea mongaensis. The flowers have the convexly-skewed orientation. **111**. T. speciosissima. The tepals are sagittally incomplete valvate and are concavely skewed. **112.** Embothrium coccineum. The tepals have a sagittally incomplete valvate aestivation pattern and the flowers of a pair are concavely skewed. **Scale bars**: 101, 109, 111-112=250μm; 102-107, 110=100μm; 108=50μm.



Figs. 4.101-4.112

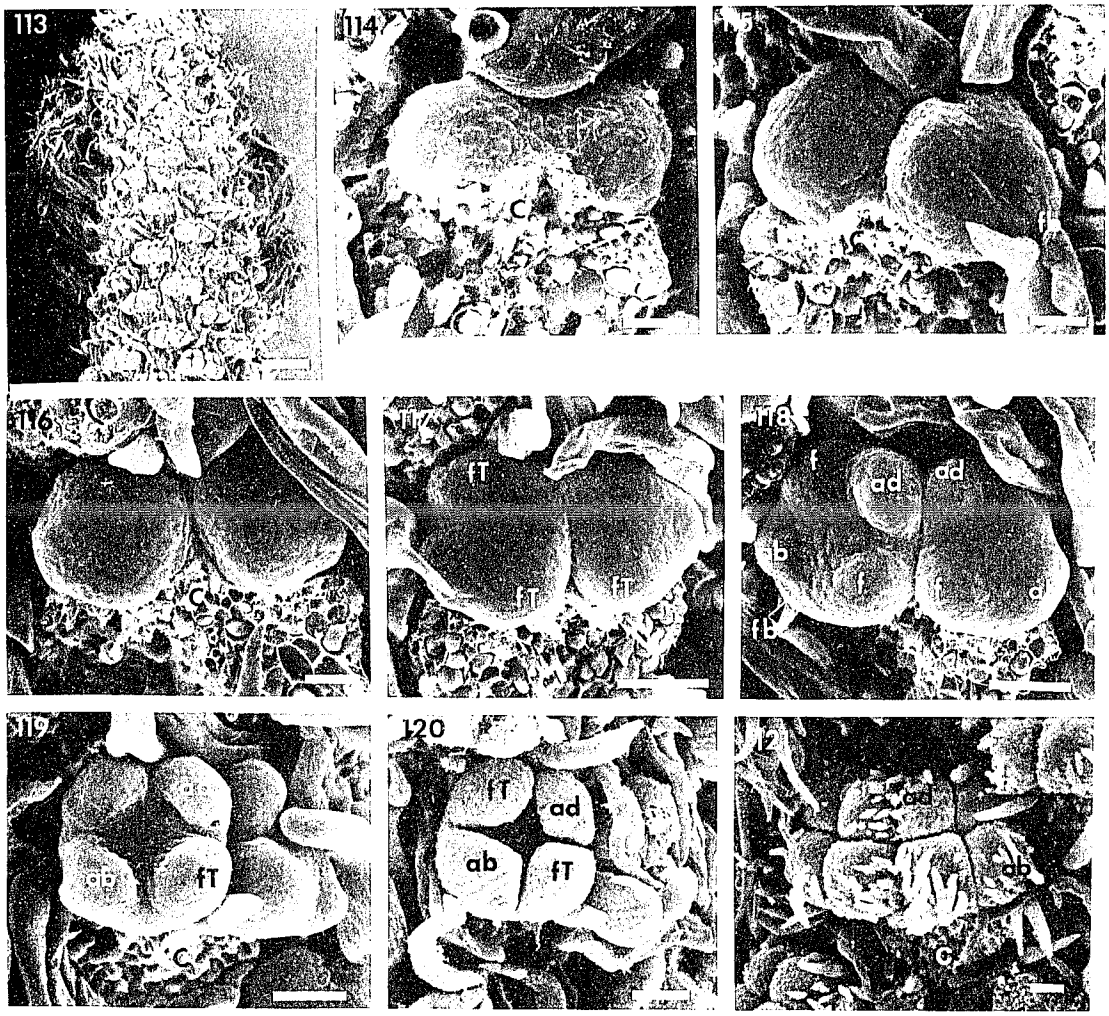
was not available. The earliest stages of development of Hakea elliptica were similar to species of Grevillea baileyana described below except that the primary axis of the inflorescences are compact and condensed and develop in leaf axils in the former.

Inflorescences of Grevillea species are extremely diverse although generally terminal and heterothetic, or simple. There is a great deal of variation among the taxa examined in: 1) the development of the floral apices (with or without subtending floral bracts), 2) the arrangement of the flower pairs (frontally bisymmetrical in G. wilsonii versus concavely-skewed in G. baileyana and G. glabella) and 3) in the order of maturation of flowers on an inflorescence (acropetal in G. baileyana, basipetal in G. glabella). The diversity of features among Grevillea and Hakea will be reported at a later time. Three species of Grevillea are described here.

Grevillea baileyana McGillivray--(Figs. 4.113-121)--Along an inflorescence axis, numerous flowers are initiated acropetally around the inflorescence apex (Fig. 4.113). Within the axil of each common bract, a laterally oblate short-shoot meristem develops (Figs. 4.113-114). Floral meristems are initiated in lateral positions on the short-shoot meristem (Fig. 4.115). In some pairs, individual floral bracts appear to be initiated (fB, Figs. 4.115, 118). The floral meristems enlarge (Fig. 4.116) and the tepals in lateral positions (in the frontal planes of the floral meristems) are initiated first (Fig. 4.117), followed by the initiation of the tepals in sagittal positions (Fig. 4.118). After initiation, the flowers of each pair twist so that their arrangement is concavely skewed in relation to the frontal plane of the pair (Figs. 4.119, 3B). The apparent rotation of the flower could be the result of differential growth of the primary axis and the common axis beneath and between the flower pairs.

There is variation in the initiation of the tepals. In some flowers, the tepals appear simultaneously. In other flowers, the first two lateral tepals appear simultaneously

Figs. 4. 113-121. Flower pair development and floral organogenesis in Grevillea baileyana. **113**. Young inflorescence with numerous flower pairs; the common bracts have been removed on the observed side. **114.** Polar view of short-shoot meristem initiation. **115**. Oblique view of short-shoot and the initiation of a floral bract and floral meristems. **116**. Enlargement of two floral apices, one on each side of the short-shoot. **117.** Initiation of the tepals; the tepals in the frontal plane are initiated first and simultaneously. **118**. Alternate initiation pattern of tepals. The sagittal tepals appear to be initiated before the frontal tepals. **119**. Polar view of left flower with all four tepals present. The flowers of the pair have rotated so that they are concavely skewed. **120.** Pre-aestivation pattern of the tepals. **121.** Polar view of flower pair showing sagittally incomplete valvate aestivation. **Scale bars**: 113=250μm; 114-116=25μm; 117-121=50μm.



Figs. 4.113-4.121

followed by the initiation of the sagittal tepals (Fig. 4.117). In other flowers, the abaxial and adaxial sagittal tepals appear to be initiated first (right flower in Fig. 4.118). It should be noted that flowers exhibiting this last pattern of tepal initiation do not have a subtending floral bract (compare the right flower with floral bract to the left flower in Fig. 4.118).

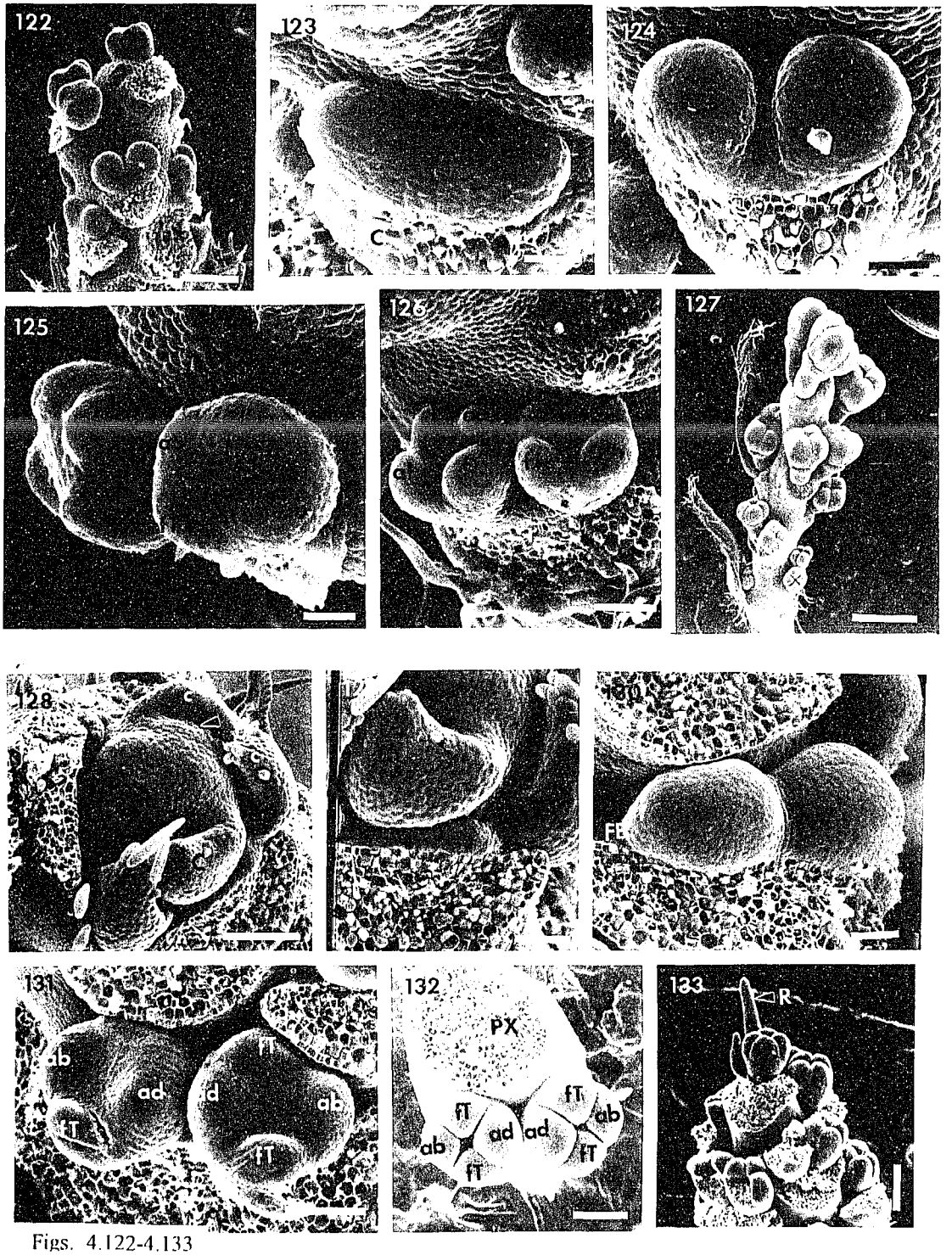
Between floral meristem initiation (note position of floral bract in Figure 4.115) and pre-aestivation (Fig. 4.120), the floral primordia rotate away from the principal axis (the left flower counter-clockwise, the right flower clockwise) and are thus concavely skewed in relation to the frontal plane of the pairs (Fig. 4.121).

Other taxa with flowers that are initiated and mature acropetally on the inflorescence axis include G. sericeae, G. vestita and Hakea elliptica.

Grevillea glabella R. Br.- (Figs.122-127) - Approximately 20-30 flowers are initiated on a principal inflorescence axis. The short-shoot meristems are initiated in common-bract axils, initiated acropetally by the inflorescence apex along the principal axis. Organogenesis of the flowers howevers occurs basipetally on the principal inflorescence axis; the tepals are initiated on the flowers distal on the inflorescence axis prior to the more basal floral meristems (Fig. 4.122). Within the axils of common bracts, bisymmetrical short-shoot meristems are initiated (Fig.123). On each side of the short-shoot meristem, a floral meristem is initiated (Fig. 4.124). The tepals are initiated simultaneously (Fig. 4.125). No floral bracts were observed. After tepal initiation, the arrangement of the flowers of a pair are concavely-skewed in relation to the frontal plane (Fig. 4.126). After aestivation, the frontal tepal tips ('ft' in Fig. 4.127) come into contact; the pattern is almost always sagittally-incomplete valvate (Fig. 4.127).

Grevillea wilsonii A. Cunn.- (Figs. 4.128-133) - The main axes of G. wilsonii have relatively few flower pairs compared to G. baileyana, and like G. baileyana are

Figs. 4.122-133. Flower pair development and floral organogenesis in two species of Grevillea. **Figs. 4.122-127.** Grevillea glabella. **122.** Principal inflorescence axis with flower pairs. The flowers on the flower pairs mature basipetally. **123** Polar view of short-shoot meristem. **124.** Polar view showing the initiation of two floral meristems, one on each side of the short-shoot. **125.** Oblique view of flower pair. The tepals are initiated simultaneously. **126.** Polar view of flowers on short-shoot showing the enlargement of the tepals. **127.** Primary inflorescence axis with flowers less mature at the base of the inflorescence compared to the upper part of the inflorescence. The tepals are sagittally incomplete valvate (arrows). **Figs. 4.128-133.** G. wilsonii. **128.** Polar view of principal inflorescence axis showing the initiation of the common bracts acropetally and the initiation of the short-shoot meristem (arrow). **129.** The initiation of the short-shoot meristem in the common bract (removed) axil. **130.** Oblique view of short-shoot. A floral bract is initiated in a lateral position on the short-shoot during the initiation of the floral meristem. **131.** Tepal initiation appears simultaneous, although the frontal tepal closest to the common bract is the largest of the four. **132.** Polar view of flower pair showing sagittally incomplete valvate aestivation. The flowers of a pair are frontally bisymmetrical. **133.** Side view of top of inflorescence showing the spicate principal inflorescence apical residuum. **Scale bars**: 122, 128, 131-132=100μm; 123-125=25μm; 126, 129-130 = 50μm; 127=500μm; 133=250μm.



Figs. 4.122-4.133

heterothetic. The common bracts (Fig. 4.128) are initiated acropetally on the primary inflorescence apex. A short-shoot meristem is initiated within the axil of each common bract (Fig. 4.129). The short-shoot meristem enlarges prior to the initiation of two floral meristems on the sides (Fig. 4.130). Small floral bract primordia become evident on the lower flanks of the enlarging floral meristems (Fig. 4.130). Tepal initiation in G. wilsonii is apparently simultaneous, although the tepals in the frontal position closest to the common bract appear largest (Fig. 4.131). After tepal initiation, the flowers of a pair are bisymmetrical. Later in development, after the development of a pedicel, each flower of a pair rotates, the left flower counter-clockwise, the right flower clockwise. The pattern of aestivation in this taxon is usually sagittally-incomplete valvate (Fig. 4.132). It should be noted that the apical residuum of the principal axis becomes elongate in this species (R in Fig. 4.133). Also, after aestivation, the floral buds enlarge in basipetal order (not illustrated).

DISCUSSION

Among taxa of Grevilleoideae it is difficult to detect the planes of symmetry in mature flowers. It is equally difficult to establish homologies among floral characters, organizations, and floral syndromes among taxa within the subfamily as well as among the other subfamilies. Johnson and Briggs (1963) claimed that "there is need for detailed and closely comparative developmental study of floral orientations" in Grevilleoideae, and emphasized that a better understanding of the morphological and phylogenetic derivation of flower pairs and floral axes in Grevilleoideae would clarify phylogenetic relationships among Proteaceae. Within an ontogenetic comparative framework, it is possible to determine the fundamental elements of inflorescence and flower organization and thus independently assess homology (Abbe, 1974, Kaplan, 1984; Tucker, 1984a, 1987a; Weber, 1988; Tucker, Douglas and Liang, 1993), because developmental stages are more straightforward and often easier to interpret than mature stages. In order to determine the

plane of symmetry and orientation of flowers among Grevilleoideae, it is essential to determine the developmental origin or basis of the flower pairs. There are three components to the ensuing discussion. Firstly, the homology of organization of the flower pairs is investigated including the **developmental basis of the flower pairs** as well as the hypothetical **phylogenetic origins** of this taxonomically defining feature. Secondly, the **developmental** stages of inflorescences and flowers of different taxa among Grevilleoideae are compared and discussed, including *short-shoot meristem development, floral bract development, tepal organogenesis and aestivation patterns.* Thirdly, the developmental events that result in **skewedness** of flowers in a pair are compared among taxa. Together, these three components are used to answer the question, is there a consistent organization of flowers among Grevilleoideae?

Nature of the flower pairs - Subfamilial differences in occurrence of flower pairs - Blastotelic inflorescences are present among all proteaceous taxa (Venkata Rao, 1965; 1971; Briggs and Johnson, 1979). In the generally racemose inflorescences of taxa of the other four proteaceous subfamilies, a single floral meristem is initiated in the axil of the first-order bract (exception in some taxa of Persoonia that have single flowers in leaf axils along flowering branches, Chapter 2). There are no subtending prophylls or bracteoles on the pedicels in these four subfamilies. In contrast, in Grevilleoideae, along the principal inflorescence axis, the flower pairs are the product of a single short-shoot meristem initiated in a bract axil (first-order meristem sensu Abbe, 1974). Two floral meristems will develop from the short-shoot meristem (each usually in the axil of a subtending floral bract), one on each side of the short-shoot meristem in the frontal plane. In this respect, the meristem is interpreted as a **short axis** (equivalent to a brachyblast sensu Hartwig, 1852 in Briggs and Johnson, 1979). The development of two flowers from the short-shoot meristem is a highly conserved ontogenetic event in all investigated

Grevilleoideae (exceptions in Lomatia and Lambertia - see below). Morphologically, a flower pair appears to represent two flowers arranged on a short secondary shoot or a two-flowered subunit (a subunit is the smallest repeating sequence of metamers (pherophyll, axillary meristem, internode) that are the product of a sylleptic axillary meristem, as opposed to a (sub)sequence that is the product of a proleptic bud; Bell, 1991; Grimes, 1992). It should be noted that the orientation of each flower in a pair is dorsiventral in relation to its subtending floral bract and/or the apical residuum (or axis) (discussed below). Each flower in a pair is a mirror image of its sister flower, including the orientation of the carpel (Douglas, Chapter 5).

Phylogenetic origin of the flower pairs - Two conditions, one flower or two flowers per bract axil, characterize different subfamilies of Proteaceae. Is one condition ancestral to the other? A significant point is that there are no transitional forms between one- and two-flowered arrangements although Venkata Rao (1971) and Johnson and Briggs (1975) have inferred a transitional form in Carnarvonia araliifolia (Carnarvonioideae) because it has only a few flowers per uniflorescence axis (Douglas, in prep.). Conflicting hypotheses concerning the derivation of the flower pairs include:

1) reduced cymoids or thyrses (dichasia with loss of the terminal flower, Haber, 1959, 1961),

2) the product of multiple origins via reduction of quaternary, tertiary and in some cases, secondary racemose inflorescence axes (Venkata Rao, 1957, 1971),

3) the product of reduction of secondary racemose inflorescence axes to two flowers (Johnson and Briggs, 1975; Venkata Rao, 1971),

4) the product of an amplification event of the axillary meristems initiated along the principal inflorescence axis.

The hypothesis by Venkata Rao (1971) and by Johnson and Briggs (1975) of reduction of secondary inflorescence axes are similar; however, Venkata Rao also asserted multiple origins of the flower pairs.

Most of the ideas surrounding the phylogenetic derivation of the flower pairs in Grevilleoideae have come from typological morphological and anatomical comparisons of mature specimens. Phylogenetic hypotheses contain implied testable ontogenetic hypotheses of morphological transitions among floral characters. A fundamental property of a phylogenetic hypothesis of morphological character transformations among taxa is the ability to logically deduce the developmental likelihood of such transformations (Kaplan, 1973; Tomlinson, 1984).

Are the flower pairs reduced dichasia? - (Fig 134) - The assertion that the flower pairs are derived from a monotelic triad (cymose dichasium or heterocladic, indeterminate thyrse, Fig. 4.134) would require suppression or loss of a terminal flower between the pair (Haber, 1959, 1961). In fact, an apical residuum of the short-shoot bearing the flower pairs persists and can bear trichomes or enlarge as a slight protuberance (rare, in Lomatia). The reduction of laterally positioned monotelic triads (dichasium) to two flowers (a monochasium) has been described in various angiosperms based on comparative studies of related taxa that maintain the monotelic triad, heterocladic thyrses, or partially cymose florescences. Examples include Papaveraceae-Fumarioideae (Parkin, 1914; Weberling, 1989), Amentiferae 'cymules' (MacDonald, 1971; Sattler, 1973; Abbe, 1974), Scrophulariaceae and Gesneriaceae (Weber, 1973), Rhamnaceae (Briggs and Johnson, 1979), Myrtales (Briggs and Johnson, 1979; Weberling, 1988) and, pseudoracemes in Papilionoideae (Tucker, 1987a; Tucker and Stirton, 1991). There are no proteaceous inflorescences observed here or described in the literature (Venkata Rao, 1965, 1971; Johnson and Briggs, 1975) that provide evidence of an anthotelic inflorescence or florescences (cymoid lateral branches). If a thyrsoid or dichasial

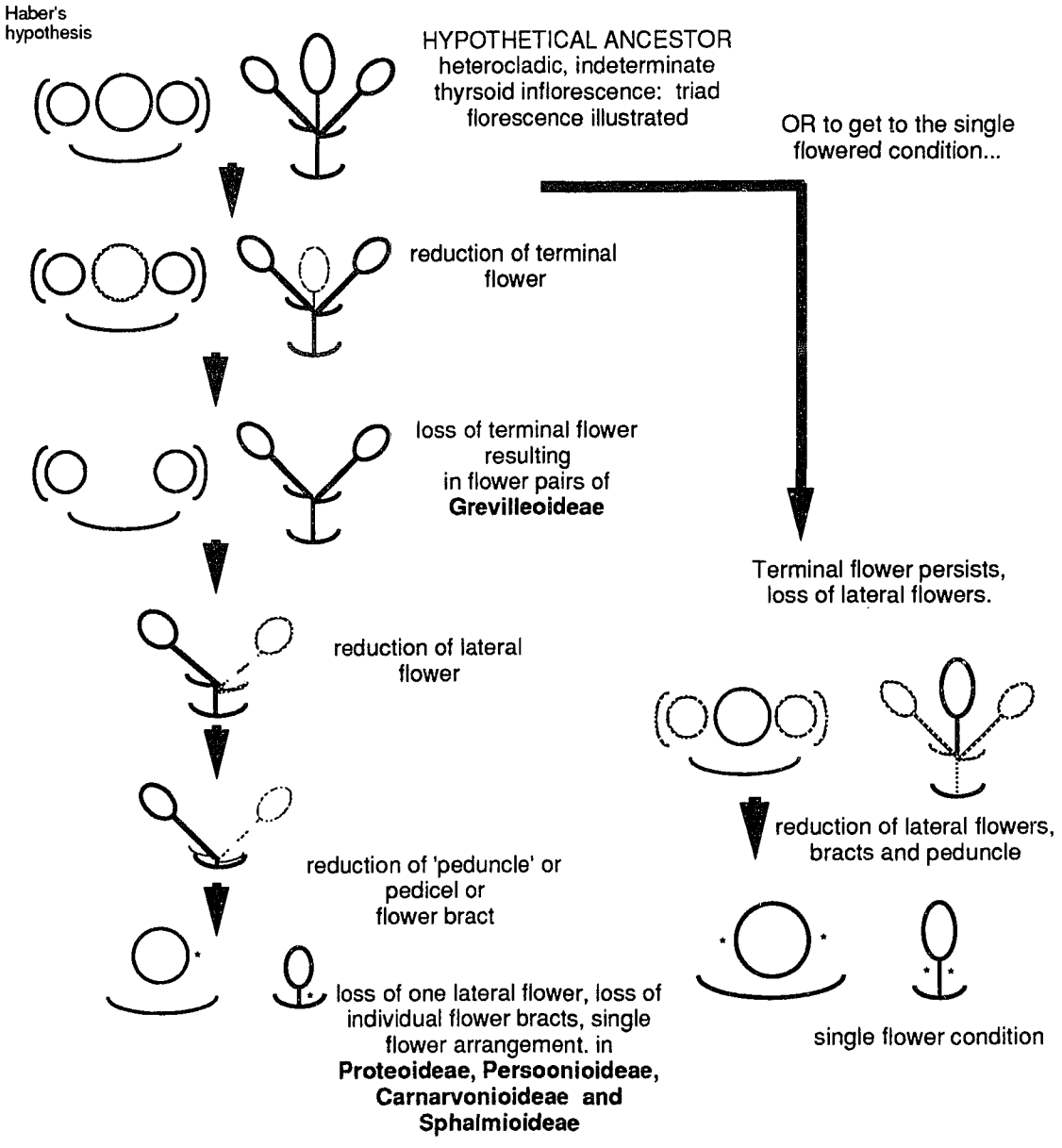

Haber's hypothesis
HYPOTHETICAL ANCESTOR
heterocladic, indeterminate thyrsoid inflorescence: triad florescence illustrated
OR to get to the single flowered condition...
reduction of terminal flower
loss of terminal flower resulting in flower pairs of **Grevilleoideae**
Terminal flower persists, loss of lateral flowers.
reduction of lateral flower
reduction of lateral flowers, bracts and peduncle
reduction of 'peduncle' or pedicel or flower bract
loss of one lateral flower, loss of individual flower bracts, single flower arrangement. in **Proteoideae, Persoonioideae, Carnarvonioideae and Sphalmioideae**
single flower condition
* = expected position of vestigial structures (i.e. bracteoles)

Fig. 4.134

condition were basal to the family, the initiation and subsequent suppression of a floral meristem between the pairs would be expected in Grevilleoideae.

Haber (1959, 1966) also hypothesized that the single-flower condition in all non-grevilleoid Proteaceae (taxa in subfamilies Proteoideae, Persoonioideae, Sphalmioideae and Carnarvonioideae) was derived from a triad ancestral type via loss of the two lateral flowers, or even reduction to one flower from the paired syndrome of Grevilleoideae (an assertion supported by Eames, 1961). All of the non-grevilleoid taxa initiate a floral meristem in a bract or leaf axil. There are no vestigial bracts or bracteoles laterally on the floral meristem before, during, or after initiation of the floral organs (Douglas, Chapters 2 and 3). In other families, the retention of suppressed features of reduced or short lateral clusters (axes) of flowers along primary axes has been demonstrated in numerous developmental studies, including the highly reduced cymule of amentiferous taxa (MacDonald, 1971; Sattler, 1973; Abbe, 1974; Kaul and Abbe, 1984), the flower clusters in Arecaceae (Uhl and Dransfield, 1984; Uhl, 1988), Hydrocharitaceae (Kaul, 1970), and Myrtales (Briggs and Johnson, 1979; Drinnan and Ladiges, 1991a). The reduction of a triad to two flowers or even to one flower has been identified in Fumarioideae (Troll, 1964 in Weberling, 1989), Rhamnaceae (Briggs and Johnson, 1979) and some species of Ononis (Papilionoideae) (Goebel, 1931). For an excellent review of presumed reduction patterns among angiosperms in general, see Parkin (1914).

Have the flower pairs been multiply derived? - Venkata Rao (1971) considered the flower pairs to be derived several times from single-flowered proteaceous taxa via reduction of a secondary racemose axis in some taxa, and by reduction of tertiary and quaternary racemose axes in other taxa. Venkata Rao's hypotheses assumed conservation of carpel orientation among Grevilleoideae, an assumption for which there is no evidence from developmental studies (Douglas, chapter 5). To support Venkata Rao's hypotheses,

ontogenetic comparisons should reveal vestigial organs, teratologies in pairing, as well as non-monophyly of either the family or of Grevilleoideae. Developmental and phylogenetic analyses (Chapter 6) do not support Venkata Rao's assertions. Although mature inflorescences are not as simple as they seem (Rickett, 1944), in cases of apparent reductions of complex branching systems such as the hypothesized reductions among Fagaceae, Juglandaceae, Myricaceae, Betulaceae and Leitneraceae (MacDonald, 1971; Abbe, 1974), Arecaceae (Uhl, 1976; Uhl and Moore, 1977, 1978; Uhl, 1988), and some papilionoid legumes (Tucker, 1987a; Tucker and Stirton, 1991), developmental investigations demonstrate the presence of vestigal structures homologous to the well-developed structures in related taxa.

Are the flower pairs reduced secondary florescence branches? - (Fig. 4.135) - The assertion that the flower pairs represent the two remaining flowers of secondary branches, and that the conflorescence (principal flowering axis with laterally attached two-flowered 'uniflorescences' of Grevilleoideae sensu Briggs and Johnson, 1979) is derived from a panicle (indeterminate raceme) (Johnson and Briggs, 1963, 1975) would require reduction of secondary axes or florescences in a compound (probably homothetic and possibly heterocladic) inflorescence to two flowers. Reduced secondary axes (dichasia, monochasia) are generally found in panicles (determinate or indeterminate), particularly at the upper nodes of the inflorescences (Troll in Weberling, 1989; Sabiaceae sensu stricto, Douglas, pers. obs.). Considering that every node along the principal axis in Grevilleoideae has a two-flowered short-shoot axis, a reduction hypothesis assumes that either fixation or canalisation of each lateral axis in first-order nodes to two flowers occurred in the proto-grevilleoid taxon. Occasionally, one flower is present in the upper bracts of the principal inflorescence axis. Ontogenetic examination reveals that the "single flowered nodes" in grevilleoids are the consequence of abortion of one side of the meristem (not illustrated). The exception is in Lambertia species examined, which

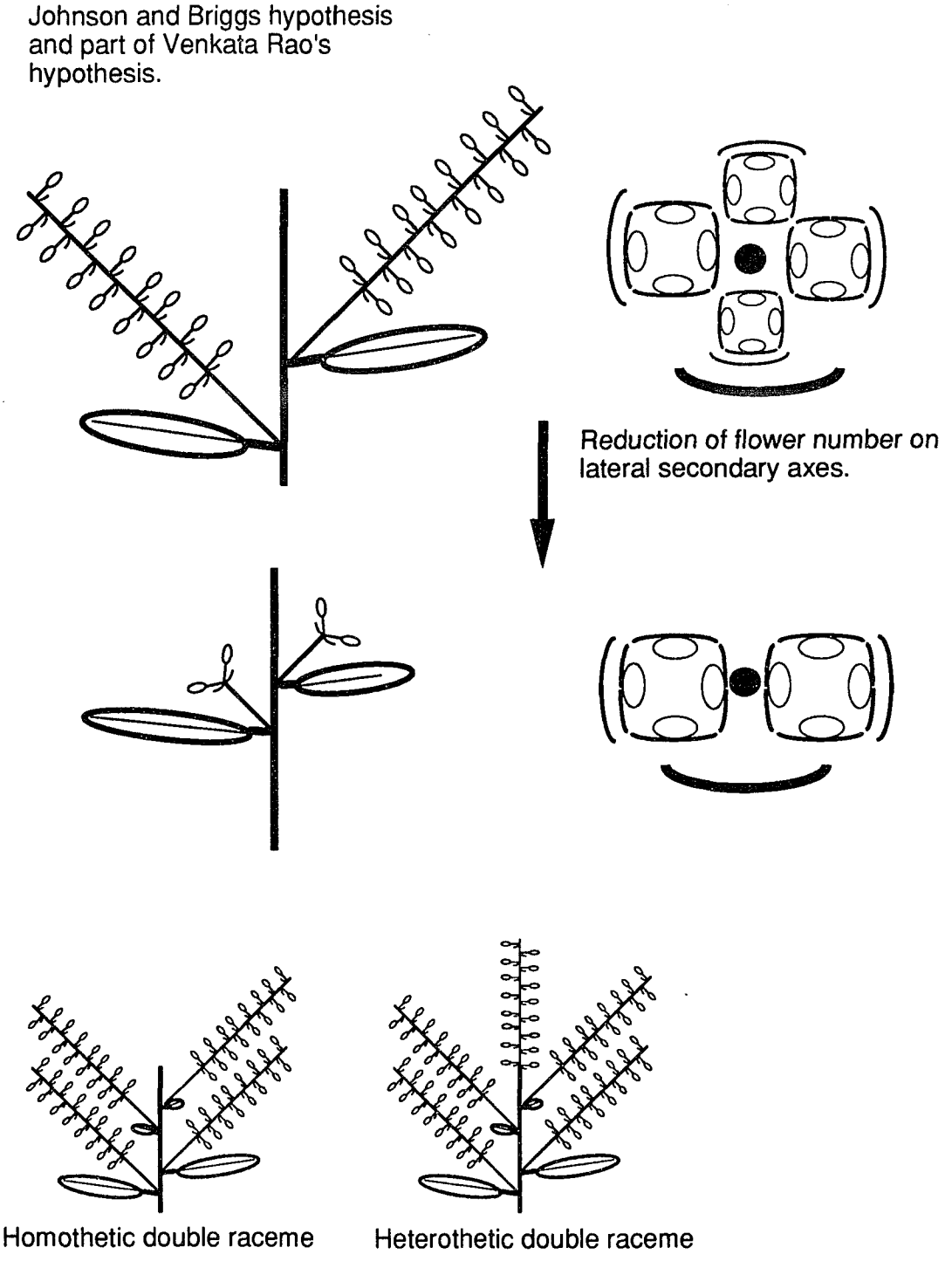

Johnson and Briggs hypothesis
and part of Venkata Rao's
hypothesis.
Reduction of flower number on
lateral secondary axes.
Homothetic double raceme
Heterothetic double raceme

Fig. 4.135

Johnson and Briggs (1975) claim shows secondary reduction, and the bases of some secondary inflorescence branches in Lomatia fraxinifolia. The developmental constraint involved with two flowers per node along a principal axis in virtually all grevilleoid taxa suggests that reduction of secondary axes must have been derived from a homothetic double raceme (sensu Weberling, 1989).

The contention of such a reduction event produces a somewhat circular argument. A reduction transformation would imply reducing a floriferous second-order axis to two flowers, and then optimizing or fixing such a reduction at each node or conserving the organization of the flower pairs along a primary axis. An examination of the adaptive significance of inflorescence structures among Proteaceae is necessary prior to any convincing evolutionary interpretation of the diversity. According to Wyatt (1982), knowledge of the adaptive significance of inflorescence structures is essential to understanding the evolutionary diversity of reproductive structures.

Could the flower pairs be the product of an amplification event? - It can be hypothesized that the flower pairs represent an amplification event of first-order meristems to short, two-flowered inflorescence axes. Such an event would result from a delay in the commitment to flowering of axillary meristems along an inflorescence. A significant point is that there are no transitional forms between one- and two flowered arrangements among Proteaceae. In this respect, the developmental events responsible for the flower pairs among Grevilleoideae are strongly conserved and demonstrate a high level of "conservation of organization' sensu Stebbins (1969: 124-125). Assumed in this hypothesis is that the inflorescence, specifically the axillary meristem, is a modifiable reproductive branching system. The concept that the inflorescence is the reproductive equivalent of a branching system was originally proposed for angiosperms by Linnaeus (1751; 1760, in Rickett, 1944). Diversity of inflorescence architecture would thus be the product of developmental shifts in timing and positions of various ontogenetic processes

starting with the products and patterns of apical meristems (Grimes, 1992). It has been hypothesized that physiological mechanisms inherent to a plant's architecture are generally present in axillary and apical meristems (White, 1979, 1984; Grimes, 1992) and that the duration and extent of growth of the meristem (that produces metamers or plant-units) is taxonomically constrained as well as ecologically influenced; conservation of form among taxa is the result. Under such a metameric concept, the inflorescence architecture of a plant is a product of conserved genetic and physiological mechanisms (developmental constraints, Waddington, 1962) inherent in the plant or more specifically, shoot, as well as the product of historically imposed phylogenetic constraints (Chevraud et al. 1983; Janson, 1992).

A corollary question in the amplification hypothesis is: when does an axillary meristem in an inflorescence become committed to a determinate floral meristem, rather than to a shoot or secondary inflorescence branch? Such a question is unanswerable from the present study. Considering that the production of two flowers per node can be viewed as a two-fold increase in reproductive effort, such a slight developmental variant could have been selectively advantageous and subsequently fixed rapidly. Amplification events leading to floriferous axes have been implied in several taxa such as Musaceae (Barker and Steward, 1962; Ram Mhan, Ram Manasi and Steward, 1962) and some legumes (Tucker, 1987a). Parkin (1914) proposed that multiflowered inflorescences are generally derived by addition of lateral flowers via axillary growth. Sundberg (1990) and Sundberg, Lafargue and Orr (in press) have proposed that there is an amplification of flower row numbers in Zea mays via a timing delay that leads to a bifurcation event of the first-order axillary meristems or "common-meristems" sensu Sundberg, 1990).

Phylogenetically, the postulation that an amplification event of a first-order meristem by a timing delay implies that the principal or primary axes of proteaceous

inflorescences are structurally homologous across subfamilial levels. This postulation will be examined within a phylogenetic context later.

From these developmental studies and other phylogenetic analyses, I would discount the theories of cymose (monotelic) reduction of Haber (1959, 1961) and those presuming multiple origins of Grevilleoideae flower pairs by Venkata Rao, as developmentally unfeasible and phylogenetically unsound. Whether the flower pairs are a result of reduction of a compound blastotelic raceme or a constrained amplification of a first-order meristem, the fact remains that a short axis is present basal to the attachment of the flowers. Also, their subtending bracts are positionally homologous to prophylls. In this sense, the flower pairs are pronodate, bearing flowers at the first two nodes of a short axis (Briggs and Johnson, 1979). Physiological and other developmental evidence is being gathered to determine the developmental origin of the flower pairs and inflorescence variation in Proteaceae, which will be reported at a later date.

Comparative early ontogeny among Grevilleoideae - The present work is not intended to be comprehensive concerning the diversity of features of grevilleoid flower pairs (for example the elongate peduncles supporting the pairs in some taxa), rather the intention is to establish the types of floral organization among the taxa and to define the developmental innovations present that give rise to some of the fundamental diversity among taxa. Developmental variations among taxa occur in the development of the short-shoot meristem and during floral organogenesis. After initiation of the **short-shoot meristem**, taxonomic variation is present in; **1)** the subsequent *enlargement of the short-shoot meristem* and, **2)** the *production and timing of individual flower bracts* (discussed below). Ontogenetic variation among taxa is also present in the earliest stages of **floral organogenesis** including; **1)** the sequential initiation of the tepals, **2)** the pattern of aestivation of the upper portions of the tepal lobes. Topological similarities among floral orientations of Grevilleoideae can be established based on the developmental comparisons.

Similarly, the skewed positions of the flowers in relation to the common bract and/or primary axis present in some taxa can be interpreted.

Figure 4.136 is a diagrammatic representation comparing the diversity of early ontogenetic processes involved with the development of flower pairs and flowers of Grevilleoideae. It should be noted that the diagram in Figure 4.136 is not meant to have any phylogenetic implications; rather, observed developmental variations are portrayed from a common developmental starting point among grevilleoid taxa, the initiation of an axillary or short-shoot meristem in the axil of a common bract.

Ontogenetic variation in short-shoot meristems - *Divergent ontogenies among short-shoot meristems* - There is variation in the enlargement of the short-shoot meristem prior to the initiation of floral bracts and/or enlargement of the floral meristems, among Grevilleoideae taxa examined. In most taxa of Grevilleoideae, the short-shoot meristem becomes frontally oblate or bisymmetrical in relation to the subtending common bract (Fig.136A). The frontally bisymmetrical nature is maintained as bracts and floral meristems develop (Fig. 4.136). In the other taxa of Grevilleoideae, the meristem becomes concave or arcuate (Fig.136 column A: Banksia, Dryandra, Austromuellera, Musgravea, Macadamia, Braebium and Virotia). An individual floral bract will develop from each side of the short-shoot meristem, although highly reduced in some taxa (Braebium and Macadamia). Proximal to each individual floral bract, a floral meristem is initiated. Each flower initiates tepals in a conserved sequence, the tepals in frontal positions are initiated first, followed by the adaxial and abaxial tepal.

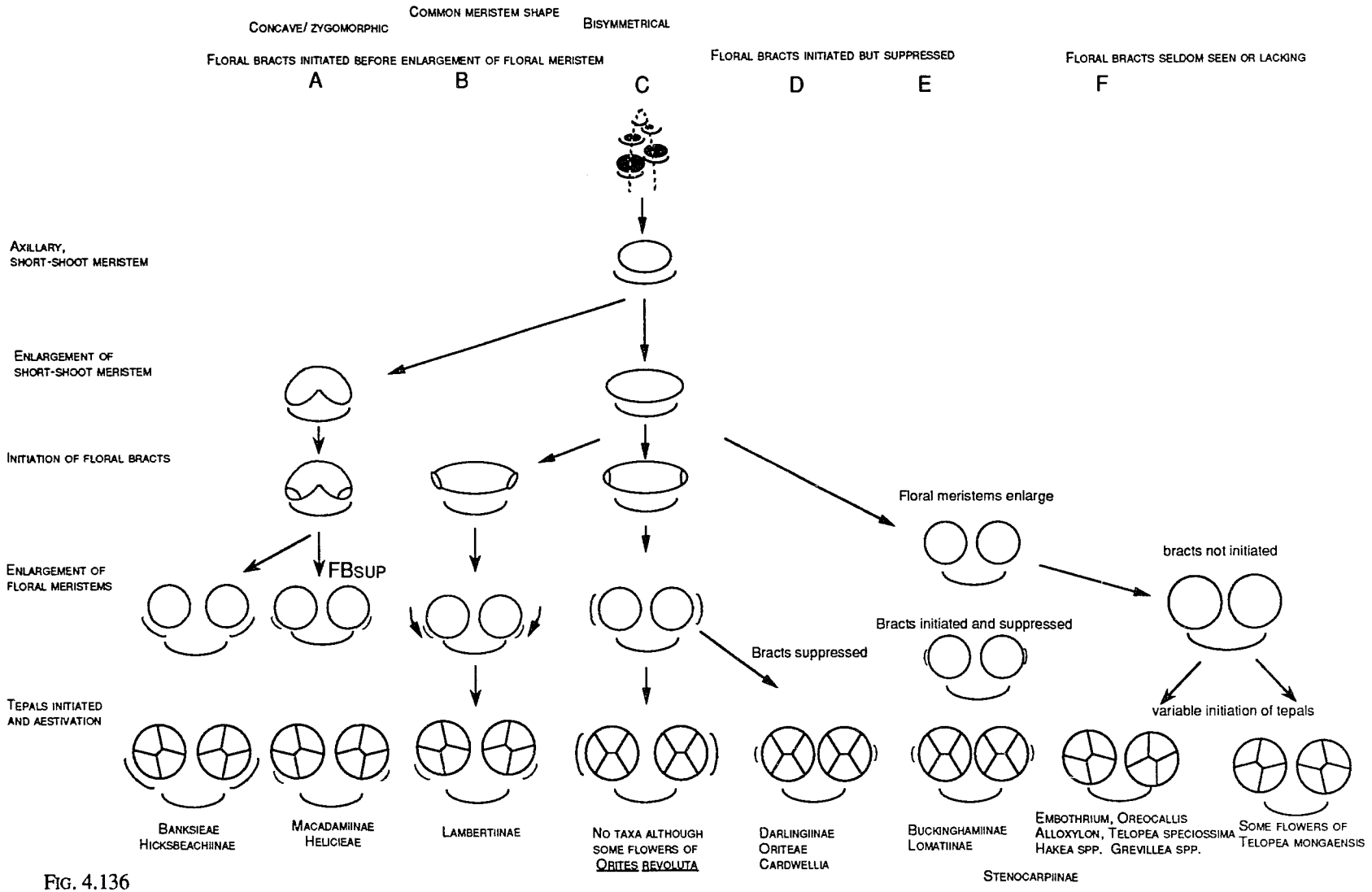

COMMON MERISTEM SHAPE
CONCAVE/ ZYGOMORPHIC
BISYMMETRICAL
FLORAL BRACTS INITIATED BEFORE ENLARGEMENT OF FLORAL MERISTEM
FLORAL BRACTS INITIATED BUT SUPPRESSED
FLORAL BRACTS SELDOM SEEN OR LACKING
A
B
C
D
E
F
AXILLARY, SHORT-SHOOT MERISTEM
ENLARGEMENT OF SHORT-SHOOT MERISTEM
INITIATION OF FLORAL BRACTS
ENLARGEMENT OF FLORAL MERISTEMS
TEPALS INITIATED AND AESTIVATION
FBSUP
Floral meristems enlarge
bracts not initiated
Bracts initiated and suppressed
Bracts suppressed
variable initiation of tepals
BANKSIEAE
HICKSBEACHIINAE
MACADAMIINAE
HELICIEAE
LAMBERTIINAE
NO TAXA ALTHOUGH SOME FLOWERS OF ORITES REVOLUTA
DARLINGIINAE
ORITEAE
CARDWELLIA
BUCKINGHAMIINAE
LOMATIINAE
EMBOTHRIUM, OREOCALLIS
ALLOXYLON, TELOPEA SPECIOSSIMA
HAKEA SPP. GREVILLEA SPP.
STENOCARPIINAE
SOME FLOWERS OF TELOPEA MONGAENSIS

FIG. 4.136

Floral bracts - There is ontogenetic variation among taxa in the *presence, position, timing*, and *development* of the floral bracts. It should be noted that in most genera, a floral bract is present in some form.

Position of the floral bracts - The position at initiation of the two floral bracts varies between lateral and oblique positions in relation to the subtending common bract (Figure 4.136 columns A-E).

Timing of the floral bracts- - The timing of floral bract initiation varies relative to the initiation and enlargement of the floral meristem. In some taxa, the floral bracts are initiated before the floral meristems (Figure 4.136 Row 2). In other taxa, the floral bracts become apparent after floral initiation, as the floral meristems are enlarging (Figure 4.136 Row 3; columns D-E). In other taxa, no floral bract primordia are seen prior to the enlargement of a floral meristem (Figure 4.136; Row 4-6). In some of the taxa that appear to lack bracts, trichomes develop in the positions where one would expect to observe bracts (Figure 4.136.row 4). These trichomes are distinctly different from the primary inflorescence axis trichomes when observed under a dissecting scope. In other taxa, bract primordia are not apparent at any stage of development (Figure 4.136 Row 5-6; Most flowers of Grevillea spp., Hakea spp., Telopea mongaensis).

Floral bract development - The floral bracts become quite prominent in size in Banksieae, Hicksbeachiinae, Heliciinae, Hollandaeiinae, and Floydiinae. In the other taxa, the floral bracts tend to be small and remain inconspicuous later in development when the flowers and inflorescence axes enlarge. Dense and elongate hairs on the primary axis, pedicels and tepals also obscure floral bracts. The presence of floral bracts in Grevilleoideae has been hypothesized as the ancestral condition among Grevilleoideae (Johnson and Briggs, 1975).

There is an association between the size of the floral bract and other features of flowers and inflorescences. That is, if a floral bract's primary function is to protect young flowers, one would expect to see an inverse relationship between the size of floral bracts and the size of the common bract that appears to serve a similar function. In most taxa that have relatively well developed floral bracts, the common bracts are generally narrow. Taxa that have small floral bracts tend to have wider (cuneate) common bracts. There is an apparent contradiction in that the taxa that lack floral bracts, like some Grevillea species and Embothriinae, similarly have narrow common bracts and in most cases also lack dense trichome cover on inflorescence axes. In these cases, the common bracts are elongate and/or the developing inflorescences are usually enclosed within a leafy involucre during early developmental stages. In other species of Embothriinae and Grevilleae, the common bracts are broad at the earliest stages of floral development. At later stages of floral development, the growth of the common bracts is arrested. Whether or not there has been a phylogenetic reduction in floral bract size, there appears to be a functional association between the development of floral bracts and the common bract. The reduction and/or eventual phylogenetic loss of an organ has generally been associated with a lack or loss of function (Goebel, 1905; Rensch, 1959; Eames, 1961; Stebbins, 1974; Gould, 1977; Tucker, 1987b). Reduction of bracts in several taxa is associated with presence of clusters of flowers along principal axes (Juglandaceae, Myricaceae, Fagaceae, Betulaceae: MacDonald, 1971; Arecaceae: Uhl and Dransfield, 1984; Uhl and Moore, 1977, for examples).

Variation in floral and tepal organogenesis - The pattern of initiation and early development of most grevilleoid flowers is generally conserved (exceptions in some Grevillea spp. and Telopea mongaensis). Conserved events include the initiation of frontal tepals followed by the initiation of the sagittal tepals, or two dimerous whorls (Douglas, Chapter 2). Stamen initiation generally

follows the same pattern as the tepals. Payer (1857) reported organogenesis in Grevillea thelemannia as following this pattern. ***Tepal initiation*** varies among the taxa examined in the relative timing of the successive tepals: the plastochron differs in length between initiation of the frontal and sagittal tepals. The arrangement at initiation of the tepals among proteaceous flowers, including Grevilleoideae shows *eprophyllate aestivation* (Weberling, 1989). Eprophyllate aestivation is the position of the first two floral organs or segments (in dicots) in flowers that lack prophylls (Weberling, 1989); a more appropriate term would probably be *eprophyllate initiation*, as the term aestivation generally defines the overlapping of floral organs in bud and does not always reflect the sequence of initiation (see below; Douglas, Chapter 2). The influence of the preceding floral bracts appears to direct the initiation and organization of tepals in grevilleoid flowers. In some cases the pattern of *aestivation* of the tepal tips varied.

Plastochron reduction - Sequential tepal initiation varies among the grevilleoid taxa examined. The variation in timing observed here is based on a general difference in the developmental intervals between the initiation of the successive tepals (plastochrons). In some taxa, the tepals are initiated successively in sub-opposite positions starting with successive initiation of the tepals in the frontal plane, and then successive initiation of the tepals in the sagittal plane. The plastochron is longer between the second and third tepal (between the frontal pair and the sagittal pair) than the plastochron between the first and second tepals (lateral positions) and the third and fourth tepals (sagittal positions). Thus, the four tepals arise as two dimerous whorls. A similar pattern of initiation was found in the flowers of taxa among Persoonioideae and Proteoideae (Chapter 2 and 3), Lauraceae (Endress, 1972), Myrtaceae (Payer, 1857; Drinnan and Ladiges, 1988, 1989a-c, 1991a-b), male flowers of Quercus (Fagaceae) (MacDonald, 1971); Urticaceae (Sattler, 1973) four-merous Papaveraceae (Sattler, 1973; Karrer, 1991), Brassicaceae (Sattler, 1973;

Endress, 1992) some Potamogetonaceae (Posluszny, 1993), Onagraceae (Sattler, 1973), and Buxaceae (pers. obs.; Drinnan, pers.comm.).

Reduction of the developmental interval between the initiation of successive tepals occurs in many of the taxa examined. The reduced plastochrons could result either in simultaneous pairs, with the frontal tepals appearing first (e.g. Banksia species); or virtually simultaneous initiation of all four tepals (some flowers of Cardwellia, Grevillea glabella). In some cases, the last tepal to be initiated is in the abaxial position (e.g. Opisthiolepis, and Darlingia). Reduced plastochrons among organs in four-merous flowers have been identified in Brassicaceae, Rhamnaceae, Oleaceae (Sattler, 1973), some Myrtaceae (Drinnan and Ladiges, 1989a), Potamogetonaceae (Posluszny, 1993), and some flowers of Amentiferae (MacDonald, 1971). The reduction in plastochrons between successive organs in the same series (i.e. sepal series, stamen series, also called whorls, Tucker, 1984a) has been identified in several angiosperm groups including Leguminosae. According to Endress (1987), there is little difference between the successive versus whorled initiation of organs, as flowers of the same taxa can do both. Although floral phyllotaxis is an extensively diverse subject with many recent papers, it is not the purpose of this paper to review that literature.

Organ influence on position of tepals - The sequential initiation of tepals in most Proteaceae with the frontal tepal pair arising first, follows what Weberling (1989; Eichler, 1875/1878) termed "eprophyllate aestivation". That is, the first two floral organs to develop are **positioned** as if they were prophylls (two in dicots, one in monocots; Weberling, 1989). Identification of taxa with flowers that have eprophyllate aestivation has been based on examinations of mature flowers (Weberling, 1989; Eichler, 1875-8). It should be noted that the position of the first two organs can change due to differential enlargement of the flower in some taxa. A developmental presumption in eprophyllate aestivation patterns is that the position at initiation of the first two primordia of a flower is

influenced by the position of the preceding organ(s), or that the sequential initiation of an organ from a meristem is influenced by the position of the preceding organ (Hofmeister, in Weberling, 1989; Eichler, 1875-8; termed phyllotactic continuity within a flower by Posluszny, 1993). In this case, the organ subtending the flower (bract or leaf in the case of blastotelic inflorescences) influences the subsequent initiation of the first floral organs; therefore a more appropriate term would be **eprophyllate initiation**. In taxa with a single flower borne in a leaf axil, the first two organs to be initiated are generally in lateral positions, analogous to prophylls on a vegetative meristem. Examples include: Drimys lanceolata, (Tucker, 1959); Pseudowintera traversii, (Sampson and Kaplan, 1970); Schisandra, (Tucker and Bourland, 1994, in press); Eupomatia, (Endress, 1980); Annona, (pers. obs.); Trochodendron and Tetracentron, (Endress, 1986); Sanguinaria, (Lehmann and Sattler, 1993); Loasaceae, (Hufford, 1988); Austrobaileyaceae, (Endress, 1980, pers. obs.); Nelumbonaceae, (pers. obs.). Similarly, the initiation of the first two floral organs in lateral positions is found in taxa with flowers borne in bract axils on blastotelic inflorescences (Papaverales, Sattler, 1973, Karrer, 1991; Peperomia (Piperaceae; Tucker, 1980, Sattler, 1973); some flowers of Scrophulariaceae, (Weber, 1973; Armstrong and Douglas, 1989); Pedicularis, (Douglas, 1989); Liquidambar styraciflua (Hamamelidaceae; Wisniewski and Bogle, 1982);, Buxaceae, (pers. obs. and Drinnan, pers. com.); Potamogetonaceae, (Posluszny, 1993); some taxa of Mimosoideae, (Ramirez-Domenech and Tucker, 1990); Sabia species, (pers. obs.); Lardizabalaceae, Coriariaceae, (pers. obs.).

A contrasting order of initiation is seen in taxa that have bracteoles (prophyllate aestivation sensu Weberling, 1989). The first floral organ (or first two floral organs in some four-merous taxa) is initiated on the side furthest from the axis and in a plane perpendicular to the transverse or frontal plane in numerous Leguminosae (Tucker, 1984a, 1987a, 1987b, 1988, 1992, Tucker and Stirton, 1991), Juglandaceae, Myricaceae,

Fagaceae (MacDonald, 1971; Sattler, 1973; Abbe, 1974); Arecaceae (Uhl, 1988), and Onagraceae (Sattler, 1973).

The sequence of initiation of the tepals in Proteaceae appears to be directly influenced by the preexisting organs, particularly a floral bract in taxa of Grevilleoideae. In Telopea mongaensis, the tepals appear to be initiated in a unidirectional sequence in some flowers; the first tepal arises on the abaxial side followed by the initiation of the frontal tepals, and lastly the adaxial tepal. Stamen initiation in Telopea mongaensis is also unidirectional. Unidirectional initiation of floral organs has been reported in Leguminosae (Tucker, 1984b, 1988, 1992), Scrophulariaceae (Armstrong and Douglas, 1989), and Reseda (Resedaceae; Payer, 1857; Leins and Sobick, 1977). Interestingly, there is no evidence of floral bracts in T. mongaensis. In some flowers, the first tepal to be initiated is in the abaxial position, analogous with the position where one would expect to see the floral bract. Some flowers of Grevillea baileyana also lack floral bracts and have a similar unidirectional sequence of initiation; the abaxial tepal is initiated first, in a position analogous with the position where one would expect to see a floral bract. There appears to be an association between the lack of a floral bract and unidirectional tepal initiation in these taxa. Tucker (1991) examined Gleditsia species that lack floral bracts. In species lacking bracts, Tucker (1991) found that the patterns of sepal initiation were chaotic and that the variation in sepal initiation affected the initiation of subsequent organs in the flower.

Aestivation patterns versus sequential tepal initiation - The most common tepal aestivation pattern among investigated taxa is sagittally incomplete valvate, in which the frontal tepal tips contact one another and the sagittal tepals fill the spaces between the lateral tepals. The generally conserved aestivation pattern appears to be a reflection of the order of initiation of the organs: the frontal tepal pair initiated prior to the sagittal tepal

pair. Other four merous taxa that have valvate perianth whorls with a similar pattern of tepal or sepal initiation include Eucalyptus, Angophora and Eudesmia in the Myrtaceae (Drinnan and Ladiges; 1988, 1989a-c; 1991a-b). Some exceptional flowers of grevilleoid taxa have simultaneous initiation of four tepals (some flowers of Cardwellia, Grevillea vestita, G. wilsonii). Historically, it has been assumed that the pattern of aestivation in flowers is a reflection of the sequential initiation of organs (Hirmer, 1918; Schimper and Braun, 1835; Weberling, 1989). Several floral developmental studies have demonstrated that aestivation patterns do not always reflect the initiation order of the perianth organs (e.g. Tucker, 1987b; Armstrong and Douglas, 1989). In many angiosperm groups, the pattern of aestivation is phylogenetically conserved at different taxonomic hierarchical levels, although usually above the tribal level (Leguminosae, Tucker, 1987b,Tucker and Douglas, in press; Scrophulariaceae, Armstrong and Douglas, 1989; Acanthaceae, Scotland, Endress and Lawrence, 1994).

Skewedness of flowers in pairs - In many Grevilleoideae, the mature flowers in a pair are skewed in relation to the common bract, the principal axis and to each other. There are three different developmental processes that can occur at different times in different organs that are responsible for the skewedness of the flower pairs. In the first case, the short-shoot meristem becomes concave or arcuate prior to the initiation of the floral meristems (Fig. 4.136, columns A-B). In the second case, differential enlargement of the primary inflorescence axis and the short-shoot can occur, resulting in the rotation of the flowers relative to the common bracts (the left flowers counter-clockwise, the right flowers clockwise). In the third case, the pedicel can rotate in later developmental stages (not illustrated here).

The pattern of aestivation in flowers of most Grevilleoideae is associated with the orientation of the the flowers. The abaxial side of the flowers is furthest away from the common origin (secondary axis) of the flower pairs. Similarly, the point of contact between the frontal tepals forms a line parallel with the median sagittal plane.

Is there a conserved organization of flowers among Grevilleoidae? - Based on the empirical evidence from this developmental morphological study, the orientation of the flowers of Grevilleoideae is highly conserved. Each flower of a pair is dorsiventrally aligned and subtended by a floral bract (most taxa) and on a short common axis. In addition, the sequence of tepal initiation is generally conserved among most taxa; the frontal tepals arise prior to the sagittal tepals. Furthermore, the orientation or skewedness of flowers in a pair among certain taxa is related to the position of the floral meristem and the differential growth of the primary axis. Thus, there is conserved organization.

Conservation of organization among flowers in inflorescences has been identified in other angiosperm groups (e.g. Leguminosae, Tucker, 1984a, 1987b, 1988, 1991; Tucker and Douglas, in press). Tucker (1984a) hypothesized that the earliest stages of organogenesis (such as sequential initiation and position at initiation) are more phylogenetically conserved than the changes that occur among taxa later in development. If the assertion of developmental conservation of early floral ontogenetic stages applies to Proteaceae, one would expect to find conservation of early ontogenetic patterns among subfamilies (as in Tucker, 1984a, 1988; Kirchoff, 1988; Armstrong and Douglas, 1989). Such conservation is present in the earliest organogenetic stages of floral development among subfamilies in Proteaceae. In both subfamilies, the frontal tepals are initiated prior to the sagittal tepals. Interestingly, there is a similar reduction of plastochrons between successive tepals in the more derived taxa of both subfamilies (sensu Johnson and Briggs, 1975; for organogenesis review, see Douglas, Chapters 2 and 3).

The position of the carpel at initiation varies dramatically in Grevilleoideae. The developmental basis of carpel orientation diversity will be examined in another paper (Douglas, Chapter 5). Among taxa of Grevilleoideae, the forms of the mature flowers are diverse. The morphogenetic events of organs vary at different taxonomic levels and will be reported elsewhere (Douglas, in prep.).

LITERATURE CITED IN CHAPTER 4

Abbe, E.C. 1974. Flowers and inflorescences of the "Amentiferae". Botanical Review 40: 160-261.

Armstrong, J.E. and A.W. Douglas. 1989. The ontogenetic basis for corolla aestivation in Scrophulariaceae. Bulletin of the Torrey Botanical Club 116: 378-389.

Barker, W. G. and F. C. Steward. 1962. Growth and development of the banana plant. 2. The transition from the vegetative to the floral shoot in Musa acuminata cv. Gros Michel. Annals of Botany 26: 413-423.

Bell, A. D. 1991. Plant Form. An illustrated guide to flowering plant morphology. Oxford University Press, Oxford.

Briggs, B. G. and L. A. S. Johnson. 1979. Evolution in the Myrtaceae - Evidence from inflorescence structure. Proceedings of the Linnean Society of New South Wales 102 (4): 157-272.

Brown, R. 1810. On the Proteaceae of Jussieu. Transactions of the Linnean Society 10: 15-226.

Carr, D. J. 1984. Positional information and plant morphology. Pages 461-486 *in* P.W. Barlow and D.J. Carr, eds. Positional Controls in Plant Development. Cambridge University Press, Cambridge.

Chevraud, J. M., J. Rutledge, and W. R. Atchley. 1983. Quantitative genetics of development: genetic correlations among age specific trait values and the evolution of ontogeny. Evolution 37: 895-905.

Crisp, M. D. and P. H. Weston. 1987. Waratahs--How many species? Australian National Botanic Gardens (Occ. Publ.) 9: 3-15.

Douglas, A. W. 1989. The ontogenetic basis of diverse floral morphologies in Pedicularis L. (Scrophulariaceae). M.S. Thesis. Illinois State University: Illinois.

Drinnan, A. N. and P. Y. Ladiges. 1988. Perianth development in Angophora and the Bloodwood Eucalypts (Myrtaceae). Plant Systematics and Evolution 160: 219-239.

Drinnan, A. N. and P. Y. Ladiges. 1989a. Operculum development in the Eudesmia B eucalypts and Eucalyptus caesia (Myrtaceae). Plant Systematics and Evolution 165: 227-237.

Drinnan, A. N. and P. Y. Ladiges. Corolla and androecium development in some Eudesmia eucalypts (Myrtaceae). Plant Systematics and Evolution 165: 239-254.

Drinnan, A. N. and P. Y. Ladiges. 1989c. Operculum development in Eucalyptus clöezmia and Eucalyptus informal subg. Monocalyptus (Myrtaceae). Plant Systematics and Evolution 166: 183-196.

Drinnan, A. N. and P. Y. Ladiges. 1991a. Floral development and systematic position of Eucalypus curtisii (Myrtaceae). Australian Systematic Botany 4: 539-551.

Drinnan, A. N. and P. Y. Ladiges. 1991b. Floral development in the 'Symphyomyrtus Group' of Eucalypts (Eucalyptus: Myrtaceae). Australian Systematic Botany 4: 553-562.

Eames, A. J. 1961. Morphology of the Angiosperms. McGraw-Hill Book Company, Inc. New York.

Eichler, A. W. 1875/1878. Blüthendiagramme II. Otto Koeltz, Eppenhain.

Endress, P. K. 1972. Zur vergleichenden Entwicklungsmorphologie, Embryologie und Systematik bei Laurales. Botanische Jahrbücher der Systematik 92: 331-428.

Endress, P. K. 1980. The reproductive structures and systematic position of the Austrobaileyaceae. Botanische Jahrbücher der Systematik 101: 393-433.

Endress, P. K. 1986. Floral structure, systematics and phylogeny in Trochodendrales. Annals of the Missouri Botanical Gardens 73: 297-324.

Endress, P. K. 1992. Evolution and floral diversity: The phylogenetic surroundings of Arabadopsis and Antirrhinum. International Journal of Plant Sciences 153 (part 2): 106-122.

Engler, A. 1894. Proteaceae. Pages 119-156 *in* A. Engler and K. Prantl, eds. Natürlichen Pflanzenfamilien. Englemann, Leipzig.

Erbar, C., and P. Leins. 1981. Zur Spirale in Magnolien-Blüten. Beiträge zur Biologie der Pflanzen 56: 225-241.

Friis, E. M. and P. K. Endress. 1990. Origin and evolution of angiosperm flowers. Advances in Botanical Research 17: 99-162.

Fuss, A. M. and M. Sedgley. 1990. Floral initiation and development in relation to the time of flowering in Banksia coccinea R.Br. and B. menziesii R.Br. (Proteaceae). Australian Journal of Botany 38: 487-500.

George, A. S. 1984. The Banksia Book. Kangaroo Press, Sydney.

Goebel, K. von. 1905, 1969. Organography of Plants, Pt. II. Special Organography. Transl. by I.B. Balfour. New York: Hafner Publ. Co.

Goebel, K. von. 1931. Blütenbildung und Sproszgestaltung. Jena.

Gould, S. J. 1977. Ontogeny and Phylogeny. Belknap Press, Harvard University Press, Cambridge, Mass.

Grimes, J. 1992. Metamerism, heterochrony, and inflorescence morphology of the Pithecellobium-complex (Leguminosae: Mimosoideae: Ingeae). Brittonia 44: 140-159.

Haber, J. M. 1959. Comparative anatomy and morphology of flowers and inflorescences of the Proteaceae I. Some Australian taxa. Phytomorphology 9: 325-358.

Haber, J. M. 1961. The comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae II. Some American taxa. Phytomorphology 11: 1-16.

Haber, J. M. 1966 Comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae III. Some African taxa. Phyomorphology 16: 490-527.

Hirmer, M. 1918. Beiträge zur Morphologie der polyandrischen Blüten. Flora: 140-191.

Hole, C. C. and R. C. Hardwck. 1976. Development and control of the number of flowers per node in Pisum sativum L. Annals of Botany 40: 707-722.

Hufford, L. D. 1988. Roles of early ontogenetic modifications in the evoluton of floral fom among Eucnide (Loasaceae). Botanische Jahrbücher der Systematik 109: 289-333.

Janson, C. H. 1992. Evolutionary constraints. Evolution 46: 136-158.

Johnson, L. A. S. 1972. Evolution and classification in Eucalyptus. Proceedings of the the Linnean Society. N.S.W. 97: 11-29.

Johnson, L. A. S. and B. G. Briggs. 1963. Evolution in the Proteaceae. Australian Journal of Botany 11: 21-61.

Johnson, L. A. S. and B. G. Briggs. 1975. On the Proteaceae--the evolution and classification of a southern family. Botanical Journal of the Linnaean Society 83-182.

Kaplan, D. R. 1973. The monocotyledons: their evolution and comparative biology. VII. The problem of leaf morphology and evolution in the monocotyledons. The Quarterly Review of Biology 48: 437-457.

Kaplan, D. R. 1984. The concept of homology and ints central role in the elucidation of plant systematic relationships. *In*: T.D. Duncan and T.F. Steussey, eds. Cladistics: Perspectives on the reconstruction on Evolutionary History. Columbia University Press, New York

Karrer, A. B. 1991. Blütentwicklung und systematische stellung der Papaveraceae und Capparaceae. Ph.D. Dissertation, University of Zurich, Zurich.

Kaul, R. B. 1970. Evolution and adaptation of inflorescences in the Hydrocharitaceae. American Journal of Botany 57: 708-715.

Kaul, R. B. and E. C. Abbe. 1984. Inflorescence archtecture and evolution in the Fagaceae. Journal of the Arnold Arboretum 65: 375-401.

Kirchoff, B. K. 1988. Floral ontogeny and evolution in the ginger group of the Zingiberales. Pages 45-56*in*, P. Leins, S. C. Tucker, and P. K. Endress (eds.), Aspects of Floral Development. Gebrüder Borntraeger, Stuttgart

Lehmann, N. L. and R. Sattler. 1993. Homeosis in floral development of Sanguinaria canadensis and S. canadensis 'Multiplex' (Papaveraceae). American Journal of Botany 80: 1323-1335.

Leins, P. and U. Sobick. 1977. Die Blütenentwicklung von Reseda lutea. Botanische Jahrbücher der Systematik. 98: 133-149.

Linnaeus, C. 1751. Philosophica Botanica. 1-362.

MacDonald, A. D. 1971. Floral development in the 'Amentiferae.' Ph.D. Dissertation. McGill University, Montreal, Canada.

Nelson, E. C. 1978. A taxonomic revision of the genus Adenanthos (Proteaceae). Brunonia 1: 303-406.

Parkin, M. A. 1914. The evolution of the inflorescence. Journal of the Linnean Society 42: 511-563.

Payer, J. B. 1857. Traité d'Organogénie comparée de la fleur. Paris: Librairie de Victor Masson.

Posluszny, U. 1993. Evolution of the helobial flower. Aquatic Botany 44: 303-324.

Ram Mohan, H. Y., Ram Manasi and F. C. Steward. 1962. Growth and development of the banana plant. 3A. The origin of the inflorescence and the development of the flowers. Annals of Botany 26: 657-673.

Ramírez-Domenech, J. I. and S. C. Tucker. 1990. Comparative ontogeny of the perianth in mimosoid legumes. American Journal of Botany 77: 624-635.

Rensch, B. 1959. Evolution above the species level. Columbia University Press, New York.

Rickett, H. W. 1944. The classification of inflorescences. Botanical Review 10: 187-231.

Rourke, J. P. 1984a. A revision of the genus Mimetes Salis. (Proteaceae). Journal of South African Botany 50: 171-236.

Rourke, J. P. 1984b. Vexatorella Rourke, a new genus of the Proteaceae. Journal of South African Botany 50: 373-391.

Sampson, F. B. and D. R. Kaplan. 1970. Origin and development of the terminal carpel in Pseudowintera traversii. American Journal of Botany 57: 1185-1196.

Sattler, R. 1973. Organogensis of flowers. A photographic text-atlas. University of Toronto Press, Toronto.

Schimper, C. and A. Braun. 1835. Vorträge über die Möglichkeit eines wissenschaftlichen Verständnisses der Blattstellung. Flora 18: 145-191.

Scotland, R. W., P. K. Endress and T. J. Lawrence. 1994. Corolla ontogeny and aestivation in the Acanthaceae. Botanical Journal of the Linnean Society 114: 49-65.

Stebbins, G. L. 1969. The Basis of Progressive Evolution. University of North Carolina Press, Chapel Hill.

Stebbins, G. L. 1974. Flowering Plants. Evolution above the species level. Harvard University Press, Cambridge.

Sundberg, M. D. 1990. Inflorescence development in Zea diploperennis in relation to other Zea species. Maydica 35: 99-111.

Sundberg, M. D., C. La Fargue and A. R. Orr. in press. Inflorescence development in the "standard exotic" maize, Argentine popcorn (Poaceae). American Journal of Botany.

Tomlinson, P. B. 1984. Homology--An empirical view. Systematic Botany 9: 374-381.

Tucker, S. C. 1959. Ontogeny of the inflorescence and the flower in Drimys winteri var chilensis. University of California Publications in Botany 30: 257.

Tucker, S. C. 1979. Ontogeny of the inflorescence of Saururus cernuus (Saururaceae). American Journal of Botany 66: 227-236.

Tucker, S. C. 1980. Inflorescence and flower development in the Piperaceae. I. Peperomia. American Journal of Botany 67: 686-702.

Tucker, S. C. 1984a. Origin of symmetry in flowers. Pages 351-394 *in* R.A. White and W.C. Dickison eds. Contemporary problems in plant anatomy. Academic Press, London.

Tucker, S. C. 1984b. Unidirectional organ initiation in leguminous flowers. Ameircan Journal of Botany 71: 1139-1148.

Tucker, S. C. 1987a. Pseudoracemes in papilionoid legumes: their nature, development, and variation. Botanical Journal of the Linnean Society 95: 181-206.

Tucker, S. C. 1987b. Floral initiation and development in legumes. Pages 183-239 *in* C.H. Stirton, ed., Advances in legume systematics, Part 3.. Royal Botanic Gardens, Kew.

Tucker, S. C. 1991. Development of the cymose inflorescence, cupulum and flower of Psoralea pinnata (Leguminosae: Papilionoideae: Psoraleeae). Botanical Journal of the Linnean Society 106: 209-227.

Tucker, S. C. 1991. Helical floral organogenesis in Gleditsia, a primitive caesalpinioid legume. American Journal of Botany 78: 1130-1149.

Tucker, S. C. 1992. The role of floral development in studies of legume evolution. Canadian Journal of Botany 70: 692-700.

Tucker, S. C. and J. Allen Bourland. In press. Ontogeny of staminate and carpellate flowers of Schisandra glabra (Schisandraceae). Plant Systematics and Evolution, Supp. 8.

Tucker, S. C. and A. W. Douglas. In press. Ontogenetic evidence and phylogenetic relationships among basal taxa in legumes. *In* I.K. Ferguson and S. C. Tucker, eds. Advances in legume systematics, Volume 5.

Tucker, S. C. and C. H. Stirton. 1991. Development of the cymose inflorescence, cupulum and flower of Psoralea pinnata (Leguminosae: Papilionoideae: Psoraleeae). Botanical Journal of the Linnean Society 106: 209-227.

Tucker, S. C., A. W. Douglas and Liang Han-Xing. 1993. The utility of ontogenetic and conventional characters in determining phylogenetic relationships of Saururaceae and Piperaceae (Piperales). Systematic Botany 18: 614-641.

Uhl. N. W. 1976. Developmental studies in Ptychosperma (Palmae). I. The inflorescence and flower cluster. American Journal of Botany 63: 82-96.

Uhl. N. W. 1988. Floral organogenesis in palms. Pages 25-44 *in* P. Leins, S.C. Tucker, and P.K. Endress, eds. Aspects of Floral Development. J. Cramer, Berlin.

Uhl. N. W. and J. Dransfield. 1984. Development of the inflorescence, androecium, and gynoecium with reference to palms. Pages 397-449 *in* R. A. White and W. C. Dickinson, eds. Contemporary Problems in Plant Anatomy. Academic Press, New York.

Uhl. N. W. and H. E. Moore, Jr. 1977. Correlations of inflorescence, flower structure, and floral anatomy with pollination in some palms. Biotropica 9(3): 170-190.

Uhl. N. W. and H. E. Moore, Jr. 1978. The structure of the acervulus, the flower cluster of chamaedoreoid palms. American Journal of Botany 65: 197-204.

Venkata Rao, C. 1957. Cytotaxonomy of the Proteaceae. Proceedings of the Linnaean Society, N.S.W. 82: 257-271.

Venkata Rao, C. 1965. Studies in the Proteaceae V. Evolution of the inflorescence. Journal of the Indian Botanical Society 44: 244-271

Venkata Rao, C. 1971. Proteaceae. Botanical Monograph #6. Council of Scientific and Industrial Research. New Delhi.

Waddington, C. H. 1962. New patterns in genetics and development. Columbia University Press, New York.

Weber, A. 1973. Die Struktur der parblütigen Partialfloreszenzen der Gesneriaceen und bestimmter Scrophulariaceen. Beiträge zur Biologie der Pflanzen 49:429-460.

Weber, A. 1988. Development, interpretation and phylogeny of the inflorescence of Epithema. Beiträge zur Biologie der Pflanzen 63: 431-451.

Weberling, F. 1988. The architecture of inflorescences in the Myrtales. Annals of the Missouri Botanical Gardens 75: 226-310.

Weberling, F. 1989. Morphology of Flowers and Inflorescences. Cambridge University Press, Great Britain.

Weston, P. W. 1983. The systematics and biogeography of the Persooniinae (Proteaceae). Ph.D. diss. University of Sydney, N.S.W., Australia.

White, J. 1978. The plant as a metapopulation. Annual Review of Ecology and Systematics 10: 109-145.

White, J. 1984. Plant metamerism. Pages 15-47 *in*: R. Dirzo and J. Sarukhan, eds. Perspectives on Plant Population Ecology. Sinauer, Sunderland, MA. .

Wisniewski, M. and A. L. Bogle. 1982. The ontogeny of the inflorescence and flower of Liquidambar styraciflua L. (Hamamelidaceae). American Journal of Botany 69: 1612-1624.

Wrigley, J. W. and M. Fagg. 1988. Banksias, Waratahs and Grevilleas. Collins Publishers, Sydney, N.S.W., Australia.

CHAPTER 5

THE DEVELOPMENTAL BASIS OF DIVERSE CARPEL ORIENTATIONS IN GREVILLEOIDEAE (PROTEACEAE)

INTRODUCTION

Among taxa of the proteaceous subfamily Grevilleoideae (41 genera), the orientation of the carpel varies. The orientation of the single carpel has been described as either in a dorsiventral orientation (the cleft aligned along the median sagittal axis) or an oblique or diagonal orientation (the cleft faceing the point of intersection between two tepals; Venkata Rao, 1957; Johnson and Briggs, 1963, 1975). In flowers of taxa from the other four subfamilies (Persoonioideae, Proteoideae, Carnarvonioideae and, Sphalmioideae; Johnson and Briggs, 1975) the carpel is consistently dorsiventrally oriented (Venkata Rao, 1957, 1971; Johnson and Briggs, 1963, 1975).

Venkata Rao (1957, 1971) suggested that there are many carpel orientations among grevilleoid taxa, and that a diagonally oriented carpel in one taxon might not be homologous with the diagonal orientation in another. Johnson and Briggs (1975) skeptically agreed with Venkata Rao's assertion, although they claimed that until a better understanding of floral orientations among grevilleoid flowers was obtained, attempts to interpret the diverse carpel orientations would be pointless. Interpretation of floral orientations among Grevilleoideae is made difficult because the flowers are borne in pairs in the axils of common bracts with the bracts helically arranged on a primary inflorescence axis. Individual flowers generally lack individual subtending floral bracts at maturity (Engler and Prantl, 1894; Johnson and Briggs, 1975; Douglas, Chapter 4). Ontogenetic comparisons (Chapter 4) of diverse taxa of Grevilleoideae indicate that each flower of a pair is usually subtended by a reduced/suppressed floral bract. Thus each flower of a pair is dorsiventrally aligned in the axil of a floralt bract, like flowers of the other four subfamilies. The flower pairs are two-flowered short-shoots (Chapter 4). The orientation of the flowers and the organization of the flower pairs revealed by developmental comparisons (Chapter 4) provide a frame of reference to resolve the questions about carpel orientations.

The diversity of carpel orientations among taxa of Grevilleoideae provides a unique opportunity to make a comparative developmental analysis. To determine the developmental basis of the diverse gynoecium orientations, I compare and describe the earliest stages of floral and carpel development of representative taxa within the family, primarily within Grevilleoideae. Using evidence from previous ontogenetic investigations of floral organizations and orientations among proteaceous flowers (Chapters 2, 3, 4), the developmental events that produce diverse carpel orientations will be described. The objectives of this study are fourfold: 1) How many orientations are there and are they taxonomically conserved? 2) What are the ontogenetic events that give rise to the diverse orientations? 3) What are the organizational and evolutionary implications of the diverse orientations? and 4) Is there any trend in the developmental events surrounding carpel organogenesis that support phylogenetic relationships among Grevilleoideae?

METHODS AND MATERIALS

Taxa included in the analysis and their provenance are listed in Table 5.1. All plant material was fixed in formalin-acetic acid-ethanol (FAA - 5 ml (37%) formalin - 5 ml glacial - 90 ml 50% ethanol) and subsequently stored in 70% ethanol. Floral material was microdissected in 95% ethanol under a Wild M5 dissecting microscope with fiber-optic illumination. Dissected materials were dehydrated through an acetone series and critical point dried with a Denton apparatus in carbon dioxide. Dried material was affixed to aluminum stubs with Photo-Mount glue (3M Inc.) or with colloidal graphite (Pelco Inc.). The material was then coated with 100-500 Angstroms of gold-palladium in a Hummer II sputter coater. Coated materials were examined with a Cambridge S-260 scanning electron microscope (SEM) at 20 or 25 kV. Images were recorded on Kodak Tri-X Pan 4x5 cut film and printed on Kodak Polycontrast Rapid III R.C. paper.

Table 5.1. Taxa and provenance. ** signifies taxa not illustrated but examined. Or. = carpel orientation. A=dorsiventral, single flowered subfamilies; B=dorsiventral, Grevilleoideae; C=lateral orientation; D=ventral-dorsal orientation; E=adaxial-lateral; F=abaxial-lateral orientation; G=distal abaxial orientation; +=variable, see text.

Or.	Taxa	Native to:	Source (s):
A	Persoonia myrtilloides Sieb. ex Schult. & Schult. F.	S.E. Australia	University of California at Santa Cruz (UCSC) arboretum
A	Symphionema montana R. Br.	S. E. Australia	R.B.G. Sydney - Mt. Annan Gardens
A	Serruria pedunculata R. Br.	South Africa	U.C.S.C. arboretum
B	Athertonia diversifolia (C.T. White) L.S. Johnson & B. Briggs	N.E. Queensland	N.E. Queensland.
B	Hollandaea sayerana (F. Muell.) L.S. Smith	N.E. Queensland	N.E. Queensland (B. Hyland)
B	**Helicia lamingtonia (F.M. Bail.) C.T. White	N. E. Queensland	N.E. Queensland (B. Gray, coll.)
C	**Triunia erythrocarpa D. Foreman	N.E. Queensland	N.E. Queensland
C	** Knightia excelsa R.Br.	New Zealand	U.C.S.C. Arboretum
C	Darlingia darlingiana (F. Muell.) L. Johnson	N.E. Queensland	N.E. Queensland; T. Irvine's private arboretum
C	**Eucarpha deplanchii Brongn. & Gris.	New Caledonia	New Caledonia (P. Weston)
C+	Floydia praelta (F. Muell.) L. Johnson & B. Briggs	N.E. Queensland	N.E. Queensland (T. Irvine, coll.)
C	Stenocarpus salignus R. Br.	Eastern and N.E. Australia	Strybing arboretum, San Francisco, California.
C	Buckinghamia celsissima F. Muell.	E. Queensland	James Cook University Arboretum, (Peter Jobson, coll.)
C	**Opisthiolepis heterophylla L.S. Smith	N.E. Queensland	N.E. Queensland, T. Irvine's private arboretum
C	**Lomatia fraxinifolia R. Br.	E. Australia	Strybing Arboretum

con'd

Or.	Taxa	Native to:	Source (s):
D	Cardwellia sublimis F. Muell.	E. Queensland	N.E. Queensland: 20 km NW of Atherton; Mt. Spec, QLD.
C	**Gevuina avellana Molina	Chile, South America	U.C.S.C. Arboretum
D	**Sleumerodendron austro-caledonicum (Brongn. & Gris) Virot	New Caledonia	New Caledonia (P. Weston coll.)
D	**Xylomelum salicinum F. Muell.	E. Australia	S.E. Queensland (T. Irvine, coll.)
D	**Bleasdalea bleasdalei (Syn. Turillia) (F. Muell.) A.C. Smith	Eastern Queensland	Eastern Queensland, Mt. Spec.
E	Embothrium coccineum Forst.	Chile, South America	U.C.S.C. Arboretum; R.B.G. Sydney (P. Weston)
E	Telopea speciosissima (Sm.) R. Br.	E. Australia	U.C.S.C. Arboretum
E	**Telopea mongaensis Cheel.	S.E. Australia	R.B.G. Sydney (P.Weston)
E	**Alloxylon flammeum P.H. Weston & M.D. Crisp	N.E. Queensland	N.E. Queensland
E	Grevillea vestita (Endl.) Meissner	S.W. Australia	U.C.S.C. arboretum
E	G. wilsonii A. Cunn.	W. Australia	U.C.S.C. Arboretum
E	G. baileyana McGillivray	N.E. Queensland	N.E. Queensland
E	G. asplenifolia Knight	E. Australia	U.C.S.C. arboretum
E	G. glabella R. Br.	W. Australia	U.C.S.C. arboretum
E	G. lavandulacea var. lavandulacea R. Br.	S. Australia	U.C.S.C. arboretum
E	Hakea myrtoides Meissner	S.W. Australia	U.C.S.C. arboretum
F	Banksia pulchella R. Br.	S.W. Australia	University of California at Santa Cruz (U.C.S.C.) Arboretum.
F	** Banksia praemorsa Andrews	S.W. Australia	U.C.S.C. arboretum

con'd

Or.	Taxa	Native to:	Source (s):
F	** Banksia ericifolia A.S. George	S.W. Australia	U.C.S.C. Arboretum
G	** Lambertia formosa Sm.	S. W. Australia	U.C.S.C. Arboretum
G	Lambertia inermis R. Br.	S. W. Australiaa	U.C.S.C. Arboretum
E+	Macadamia integrifolia Maiden & Betche	E. Australia	R.B.G. Sydney; U.C.S.C. arboretum; University of Melbourne; Waimea Arboretum, Hawaii (coll.); R.B.G. Melbourne; T. Irvine's private arboretum
E+	Macadamia tetraphylla L.A.S Johnson	E. Australia	R.B.G. Sydney; University of Melbourne.
E	Brabejum stellatifolium L.	South Africa	Kirstenbosch Gardens, S. Africa (T. McLellan, coll.. and D. Keats, coll.; R.L. Chapman, coll.)

Carpel orientations in 31 genera (41 species) of Grevilleoideae were investigated. Some sampling limitations resulted in incomplete stages for certain taxa (* in Table 5.1).

Measurements - Floral meristems were measured (Fig. 5.1) during organogenesis until the stage of carpel cleft formation. Absolute values were measured with the SEM. All measurements were made with a fully saturated filament at 25 kV and a working distance between 10-25 millimeters. Measurements were also made with the dissecting microscope. Meristem size was measured along the median frontal plane and the median sagittal plane. The two values were used to create a relative ratio (frontal/sagittal) and the product of the ratio (*ratio value*) calculated in order to compare different taxa. When the ratio value of the meristem is at 1.0, the meristem is referred to as actinomorphic; when the ratio value is greater than 1.0, the meristem is frontally bisymmetrical; when the ratio value is less than 1.0, the meristem is referred to as sagittally bisymmetrical. During the conversion of the floral apical meristem to carpel primordium, the height increases. Height measurements were also recorded on the SEM.

Terminology (Figs. 5.2-3) - There are different terms associated with the different planes in flowers and structures. Terminology for the two planes include the **median sagittal** plane that bisects a flower, its subtending leaf (or bract) and the inflorescence axis into two mirror-image halves, and the **median frontal** plane, perpendicular to the first plane. The **adaxial** side of the flower is the upper half, closest to the inflorescence axis, and the **abaxial** side is the lower half of the flower closest to the subtending leaf (or bract). Because the carpel orientation varies among Grevilleoideae, the lateral sides of the flower are separately defined. The side closest to the common bract (first-order bract) of a flower pair is referred to as the **common-bract side**. The side of a grevilleoid flower closest to the primary inflorescence axis is referred to as the **primary-inflorescence side**.

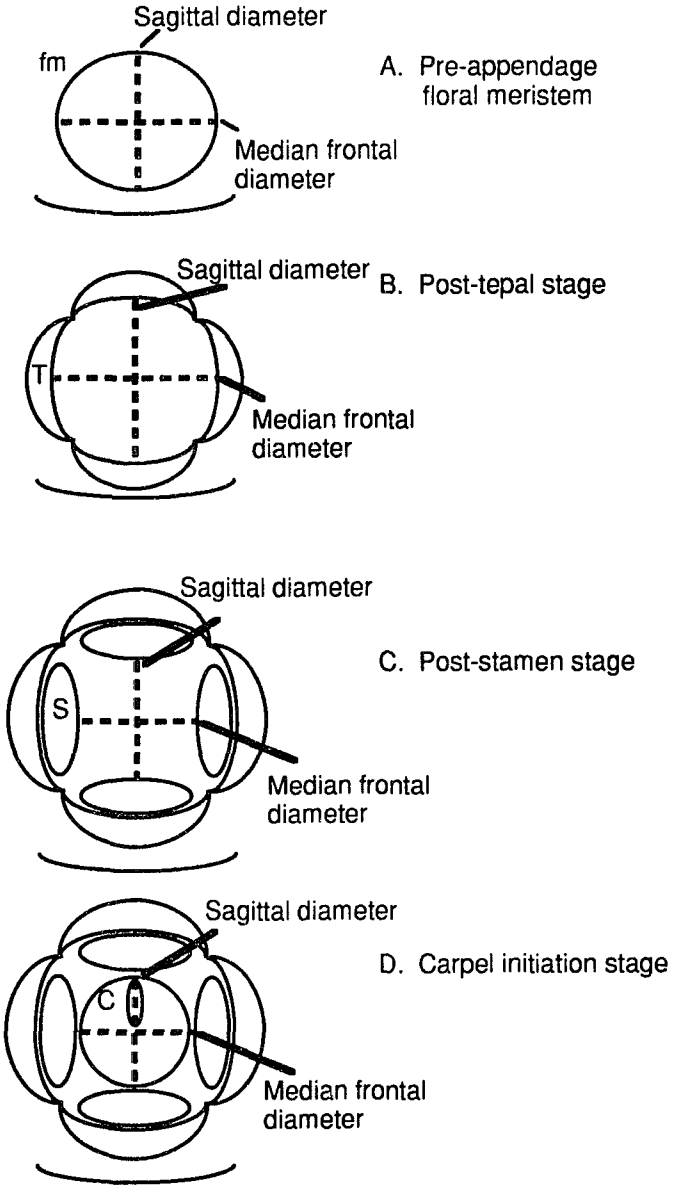


Figure 5.1. Diagram illustrating measurements of floral meristem at different stages of organogenesis including early carpel development.

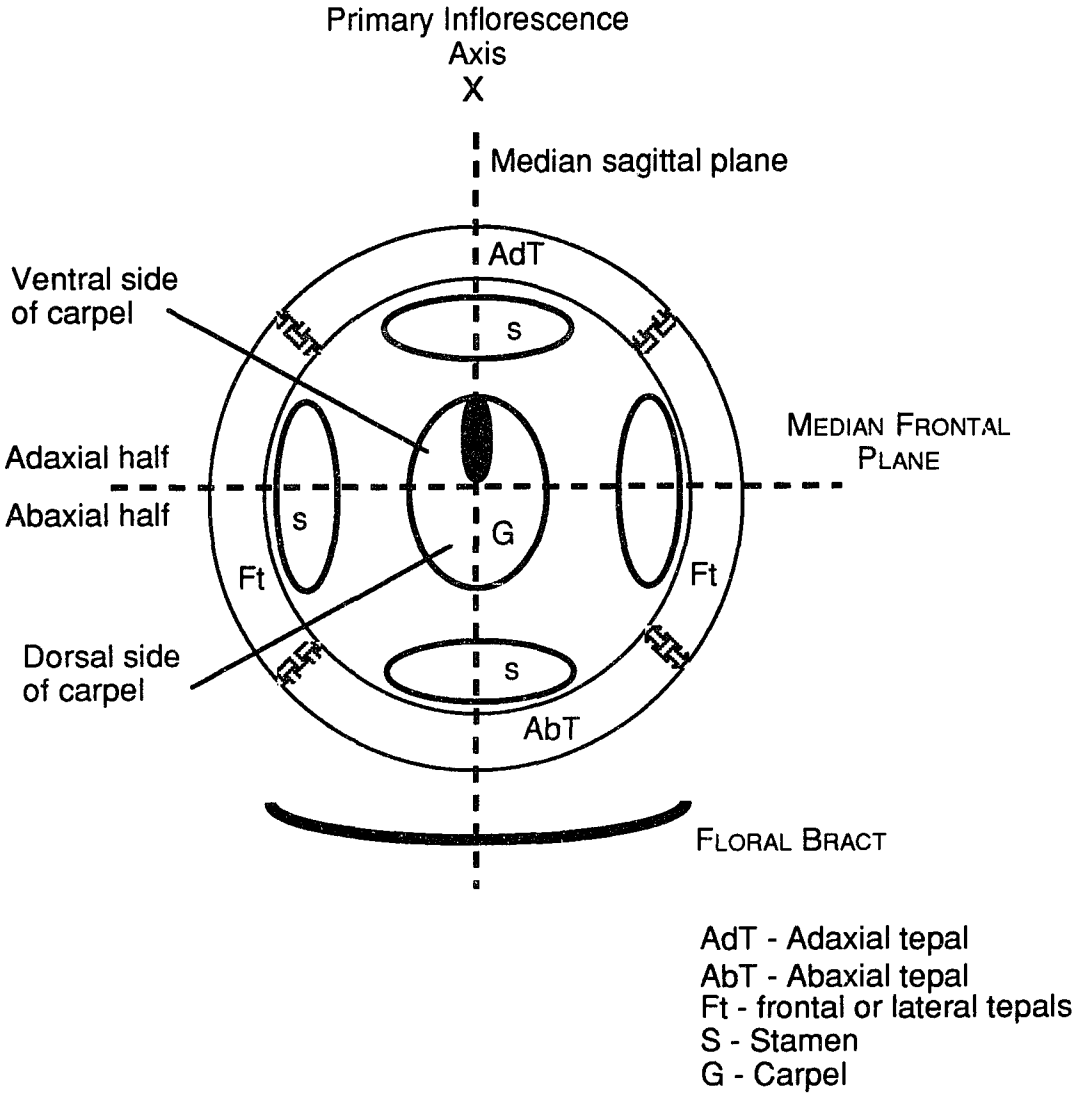
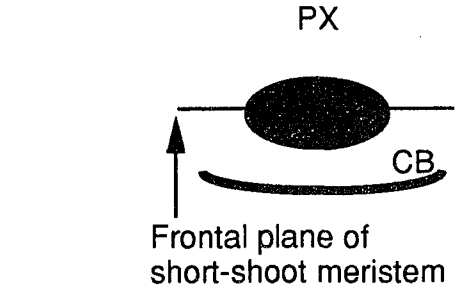
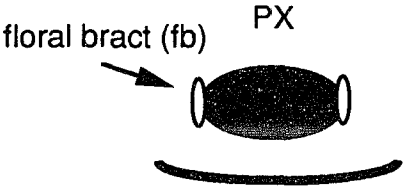


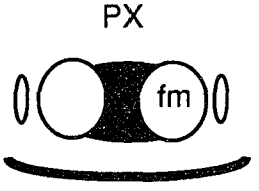
Figure 5.2. Floral groundplan of proteaceous flower including planes of symmetry



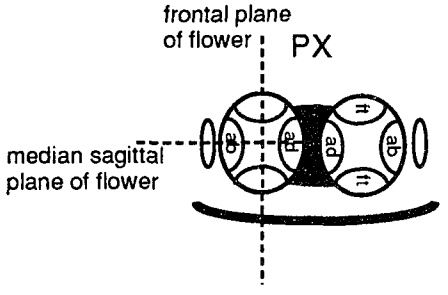
A. Axillary or short-shoot meristem initiated in axil of common bract (CB)



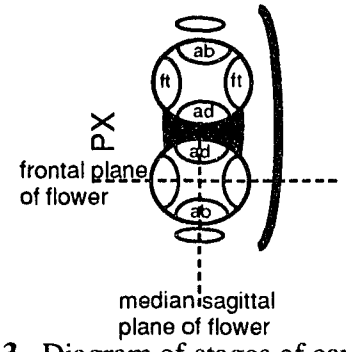
B. Floral bracts initiated, one on each side of short-shoot meristem in frontal plane



C. Floral meristems develop on each side of short-shoot meristem in axil of floral bract if present



D. Floral meristems enlarge and tepals initiated; note planes of the flowers



E. Rotated view showing planes of flowers. The upper flower in this image (the right flower above) is a mirror image of the sister-flower.

Figure 4.3. Diagram of stages of early floral organogenesis among Grevilleoideae including the development of two flowers from the short shoot (Chapter 4).

There are different terms associated with the symmetry of flowers. **Actinomorphy** is synonymous with radial symmetry, in which the flower has multiple planes of symmetry; **bisymmetry** refers to structures that have two planes of symmetry, in which each bisects the structure or flower into two mirror-image halves (Weberling,1989; Friis and Endress, 1990), and **zygomorphy** (or **dorsiventral symmetry**) refers to structures that have one plane of symmetry, the median sagittal plane. In the last, the abaxial and adaxial halves are not mirror-images (Weberling, 1989; Friis and Endress, 1990). In some taxa, the flowers are zygomorphic but the carpel is upside-down; this condition is termed **ventral-dorsal symmetry** (Douglas, 1989). When a flower or structure has no plane of symmetry it is **asymmetric**.

Floral groundplan (Fig. 5.2) - With rare exceptions, flowers of Proteaceae are four-merous. The flowers have a single whorl of four valvate tepals. The term tepals is used because the phylogenetic origin of the single perianth whorl in Proteaceae is uncertain, although Proteaceae is hypothetically derived from a monochlamydeous ancestor (pre-Rosidae,Johnson and Briggs, 1975; Douglas, Chapter 2). Each flower has four stamens, one opposite each tepal (**superposed** or **antetepalous**). The stamens have tetrasporangiate anthers and basifixed filaments. Each stamen is generally **adnate** (via zonal growth) to the opposing tepal lobe (**epitepalous**).

In most proteaceous taxa, the entire floral apex, after stamen initiation, is utilized in the formation of the carpel and, thus the carpel is termed a **terminal** carpel. In other taxa investigated, the carpel forms in a **lateral** position in relation to the longitudinal axis. During **terminal** carpel inception, there is ambiguity as to when the remaining floral meristem becomes a carpel primordium. From a histogenetic study of terminal carpel inception in flowers of Persoonioideae (Douglas, Chapter 2), there is a correlation between cellular configuration changes in the meristem and the morphological expression

of zygomorphy. For present purposes, the carpel is considered present when zygomorphy is expressed in the floral meristem by cleft formation.

There are different sides of a carpel (Fig. 5.2). In apocarpous taxa (having free carpels), the back side, homologous to the abaxial side of a leaf, is called the **dorsal** side of the carpel (Bailey and Swamy, 1951; Periasmy and Swamy, 1956). The opposing side is called the **ventral** side and it is on the ventral side, that the **cleft** will develop. Although adaxial and abaxial could be used for the different sides of the carpel, it is easier to describe and visualize the organization of the single proteaceous carpel in relation to the floral planes if separate terms are used.

General organography of Grevilleoideae (Fig. 5.3) - In Grevilleoideae, two flowers are present in the axil of each common bract. There are numerous common bracts on a principal inflorescence axis (conflorescence sensu Briggs and Johnson, 1979). Developmentally, an axillary meristem is initiated in the axil of the common bract (Fig. 5.3A). The axillary meristem, subtended by the common bract, is referred to as a short-shoot meristem. Usually two floral bracts are initiated, one on each side of the axillary meristem (Fig. 5.3B). The two flowers of a pair are initiated by the short-shoot meristem, each one in the axil of a floral bract if present (Fig. 5.3C). Each flower of a pair, including the individual floral bract if present, is a mirror image of its sister flower, both morphologically and during each stage of development (Fig. 5.3D-E). Developmentally, each flower is dorsiventrally aligned (Fig. 5.3D-E), usually in relation to a floral bract, so that its median sagittal axis bisects the adaxial and abaxial tepals (Douglas, Chapter 4).

Tepal organogenesis of the flowers in Grevilleoideae (Chapter 4) is similar to that of the flowers in Persoonioideae (Chapter 2) and Proteoideae (Chapter 3). The lateral tepals are initiated prior to the sequential initiation of the sagittal tepals. The sequential

initiation of tepals in some taxa is difficult to determine due to compression of the floral meristems by the common bracts and/or primary inflorescence axis.

Stamen initiation in Grevilleoideae is similar to that of other Proteaceae although precocious epitepaly complicates dissection and intepretation of stamen development. This subject will be described and discussed for Grevilleoideae at a later time.

OBSERVATIONS

Carpel orientations among Proteaceae have been described as dorsiventral or diagonal (Haber, 1959, 1961, 1966; Venkata Rao, 1957, 1971; Johnson and Briggs, 1963, 1975). Present ontogenetic investigations reveal six orientations (Fig. 5.4 A-G):

A-B. the **dorsiventral** carpel orientation (the cleft faces the *adaxial* tepal and is oriented in the median sagittal plane) is common among all subfamilies (Fig. 5.4A-B) and is predominant in most flowers of Proteoideae, Persoonioideae, Sphalmioideae and Carnarvonioideae. Among Grevilleoideae, there are five additional types of orientation.

C. the **lateral** type, defined by the carpel cleft facing the lateral tepal on the primary inflorescence side (Fig. 5.4C) the carpel cleft is aligned on the median frontal plane of each flower).

D. the **ventral-dorsal** type, defined by the cleft facing the *abaxial* tepal (Fig 4D).

E. the **adaxial-lateral** type when the carpel cleft faces the alternitepalous site between the adaxial and lateral tepal closest to the primary inflorescence axis (Fig. 5.4E).

F. the **abaxial-lateral** type when the cleft faces the alternitepalous site between the abaxial tepal and the lateral tepal closest to the primary inflorescence axis (Fig. 5.4F).

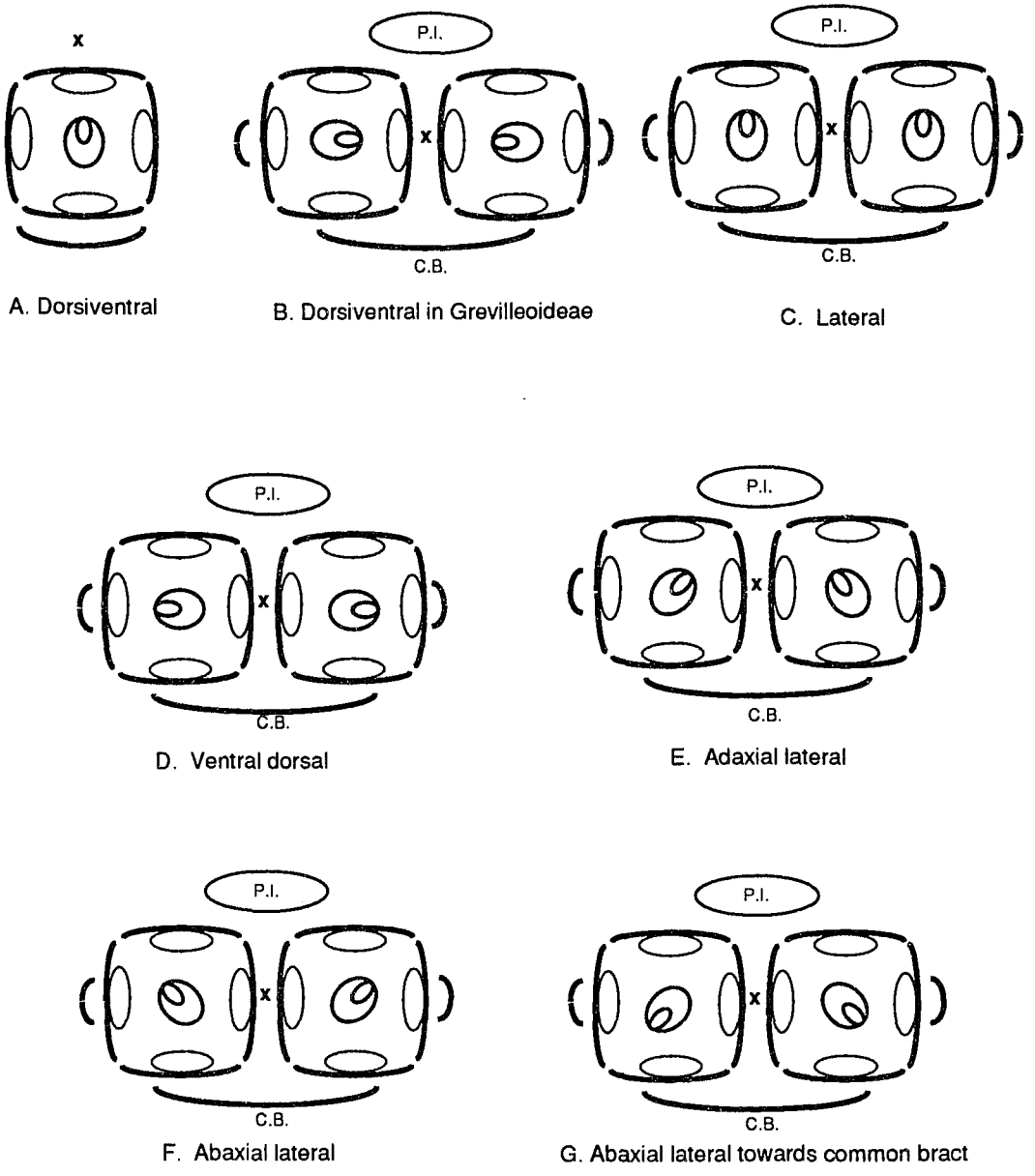


Figure 5.4. Six carpel orientations of Proteaceous flowers, particularly among Grevilleoideae B-G.

G. the **distal abaxial** type when the cleft faces the alternitepalous site between the abaxial tepal and the lateral tepal on the common bract side, away from the primary axis (Fig. 5.4G).

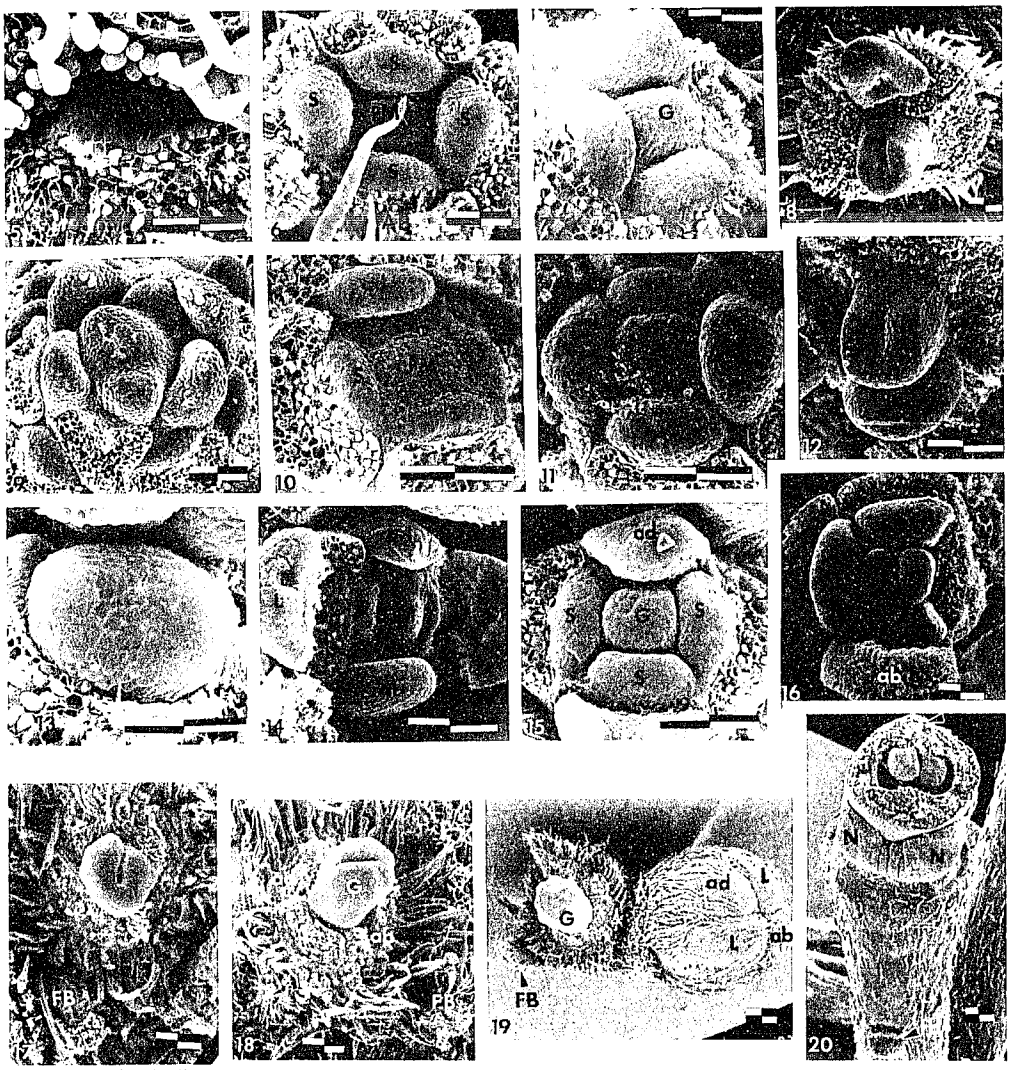
The developmental events that produce the different carpel orientations in representative taxa are described below. Included for comparison is the dorsiventral orientation of carpels in representative taxa of Persoonioideae and Proteoideae. The orientations of carpels in taxa not illustrated below are summarized in Table 5.1.

Dorsiventral orientation - (Figs.4A-B; 5-20) - Although most of this chapter deals with Grevilleoideae, taxa from other subfamilies are described and shown first for comparison. Illustrated taxa include Persoonia myrtilloides of Persoonioideae and two members of Proteoideae, Symphionema montana of the tribe Conospermeae and Serruria pedunculata of the tribe Proteeae. Flowers of taxa in these subfamilies are subtended by a leaf (Persooniinae) or bract (Proteoideae) and have a dorsiventrally aligned carpel (Fig. 5.4a, Chapter 3).

Selected stages of ontogeny are presented, with emphasis on size of the floral apex through cleft formation. In all flowers examined, a laterally oblate floral meristem develops in the leaf or bract axil (Figs. 5.5, 9, 13). Prior to tepal initiation in P. myrtilloides, the floral meristem (Fig. 5.5) is approximately 150 μm frontally wide by 70 μm sagittally long (ratio value=2.14). Prior to tepal initiation in Serruria, the floral meristem (Fig 9) is approximately 190 μm x 80 μm (ratio value=2.37). Prior to tepal initiation in Symphionema, the floral meristem (Fig. 5.13) is approximately 165 μm x 100 μm (ratio value= 1.65). Tepal initiation is not shown here and have been described in Chapters 2 and 3. The first two tepals are initiated successively on the lateral sides, right or left tepal first, the opposite tepal second. In most cases, variation between the first tepal initiated is present on the same plant . The third and fourth tepals in the sagittal plane

Figs. 5.5-20. Floral and carpel organogenesis in taxa with dorsiventral carpel orientations. In all images, the flowers are aligned so that the abaxial side is on the lower side of the figure unless otherwise mentioned. **Figs. 5.5-8, Persoonia myrtilloides. 5.** Floral meristem initiated in leaf axil (removed). **6.** Early expansion of the floral meristem between the four stamen primordia (S), the tepals (T) have been removed. **7.** Oblique view of the floral meristem/carpel primordium prior to the formation of the cleft. **8.** Polar view of carpel primordium; the cleft in the adaxial half of the flower does not extend to the base of the carpel. **Figs. 5.9-12. Serruria pedunculata. 9.** Oblique polar view of terminal inflorescence showing the anthotactic arrangement and the initiation of floral meristems in bract axils. **10.** Floral meristem (FM) after stamen initiation. **11.** The floral apex enlarges and increases in height. **12.** The formation of the cleft on the carpel primordium adaxially. **Figs. 5.13-16. Symphionema montana. 13.** Floral meristem before organ initiation. **14.** Oblique frontal view of young flower after stamen initiation; the floral apex is enlarging. **15.** Polar view of flower with carpel primordium. **16.** The formation of the cleft on the carpel adaxially. **Figs. 5.17-20.** Dorsiventral orientations of flowers in Grevilleoideae. **Figs. 5.17-19.** Various views of carpel orientation in **Athertonia diversifolia. 17.** Dorsiventral polar view of young carpel. The stamens and tepals have been removed and the floral bract (FB) is shown. This is also a lateral view of the flower pairs; only the left flower shown. **18.** Side view of young carpel showing floral bract and the scars of the removed tepals and stamens. **19.** Polar view of flower-pair. The common bract has been removed (arrow head). In the dissected left flower, the orientation of the carpel is illustrated. In the right flower, the aestivation pattern of the tepals is sagittally incomplete valvate. **20. Hollandaea sayerana**. Older left flower of a pair, the carpel has been broken approximately halfway down the ovary, showing the cleft/suture and two of the numerous ovules inside the locule. On the pedicel, the floral bract is reduced in size. Surrounding the ovary is a four-lobed nectary (n). **Scale bars** = 100μm in Figs. 5.5-18; 500 μm in Figs. 5.19-20.

Symbols used for subsequent images include: L=frontal tepal; ad (AD)=adaxial usually referring to a tepal or stamen; ab (AB)=abaxial, usually referring to the position of a tepal or stamen; X=axis; S=stamen; N (n) = nectary; fb (FB)= floral bract; cb (C) = common bract; fm=floral meristem; G=carpel; FX=median sagittal floral axis; R=residuum.



Figs. 5.5-5.20

are successive as well. The stamen primordia are initiated in a similar sequence as the tepals; the two stamen primordia on the lateral sides are initiated first followed by the successive initiation of the adaxial and abaxial stamens; each stamen is superposed to a tepal lobe (Figs. 5.6, 10, 14).

Following stamen initiation, in all three taxa, the remaining floral apex is frontally broad (Figs 6, 10, 14). The meristem ratios are 100 μm x 80 μm in Persoonia (ratio value = 1.25); 80 μm by 67 μm in Serruria (ratio value= 1.2); and 65 μm x 55 μm in Symphionema (ratio value = 1.18). In all taxa, the floral apex enlarges and increases in height during the conversion to a carpel primordium.

Prior to the presence of the cleft, the carpel primordium/floral apex is a frontally broad and bisymmetrical dome (Figs. 5.7, 11, 15) The meristem ratios are 120 μm x 100 μm (ratio value = 1.2) and 65 μm high in Persoonia; 85 μm x 76 μm (ratio value 1.12) and 50 μm high in Serruria; and 77 μm x 67 μm (ratio value = 1.15) and 50 μm high in Symphionema.

I have used cleft presence as the first sign of carpel primordium morphogenesis (Figs. 5.8, 12, 16). In all taxa examined, the cleft arises adaxially and does not extend to the carpel base.

Variation was found in the carpel orientations of some flowers of species of Proteoideae. In Stirlingia latifolia and Cenarrhenes nitida, the carpels of some flowers on an inflorescence or all flowers on an inflorescence were lateral in the former or diagonal between an adaxial tepal and a lateral tepal in the latter (Douglas, Chapter 3). Venkata Rao (1961, 1971) reported similar variability in his examinations of Cennarhenes nitida.

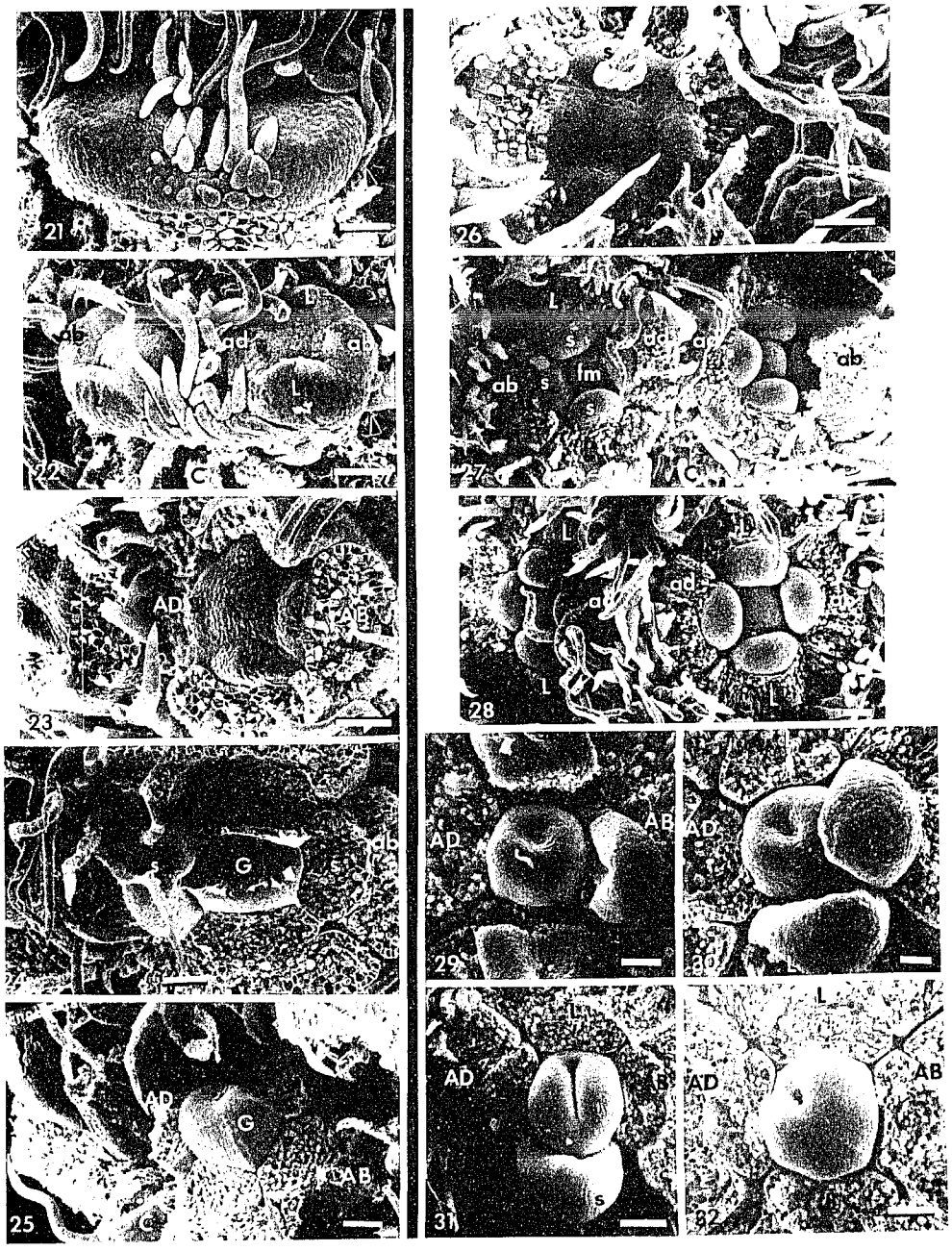
Among flowers of subfamily Grevilleoideae, dorsiventral orientation (Fig. 5.4B) appears consistently in the examined flowers of Hollandaea sayerana (Fig. 5.20, bottom

arrowhead on floral bract and top arrow head pointing to carpel suture; Helicieae). It appears in 65% of the flowers of Athertonia diversifolia (Figs. 5.17-19; Macadamieae). The other 35% of 40 flowers examined were either "ad-lat" or "ab-lat" diagonal. No young material was available for developmental comparisons.

Lateral orientation (Figs. 5.4C; 21-42) - The lateral orientation of the carpel is found in species distributed sporadically among Grevilleoideae including Neorites (Oriteae), Triunia and Helicia (Helicieae), all three genera of Knightiinae, Floydia (Macadamieae), and Stenocarpus, Opisthiolepis, Lomatia, and Buckinghamia (Embothrieae). In these examined taxa, the carpel cleft faces the lateral tepal closest to the primary inflorescence axis (Fig. 5.4C). Among the taxa, there are differences in the utilization of the floral meristem. In the first type, the carpel forms from the entire floral apex **(terminal inception)**; in the second type, the carpel forms on one side of the floral apex, leaving an apical residuum **(lateral inception).**

Terminal inception (Figs. 5.21-32) - Darlingia darlingiana (Knightieae-Knightiinae) (Figs. 5.21-25) - From the flanks of the inflorescence apex, common bracts are initiated. An axillary meristem is initiated in the axil of each common bract. From the lateral sides of the meristem, two floral meristems are initiated (Fig. 5.21) that individually have a ratio value of approximately 1.1-1.2. In most flowers, a subtending floral bract is not evident although a region of different-textured hairs develops in the area where one would predict a bract to occur. Tepals are initiated successively, the laterals appearing first followed by the sagittal pair (Fig. 5.22). After tepal initiation, the floral apex has a ratio value of 0.86-0.9. After stamen initiation (Fig. 5.23), the ratio value of the floral apex is approximately 0.8-0.86. The remaining floral meristem (Fig. 5.24), has a ratio value of approximately 0.8 and is sagittally oblate. The carpel cleft (Fig. 5.25) forms on the ventral side of the sagittally bisymmetrical carpel primordium when the primordium is approximately 85 μm high.

Figs. 5.21-32. Carpels that are laterally oriented among Grevilleoideae with ***terminal inception.*** In all images, the common bract to the flower pairs (two-flowered short-shoot) is at the bottom; thus the median sagittal plane of each flower is sideways (see Figure 5.3D-E for reference). **Figs. 5.21-25.** Floral development in **Darlingia darlingiana**. **21**. Two floral meristems are present in lateral positions on the short-shoot. **22.** The four tepals have been initiated on each flower of a pair. **23**. Slightly oblique view of the right flower of a pair after the four stamens have been initiated in antetepalous positions, the tepals have been removed. The floral apex is sagittally oblate. **24**. Polar view of right flower of a pair showing the early inception of the carpel primordium. **25.** An oblique view of the formation of the cleft on the carpel in the right flower of a pair. The cleft faces the lateral stamen and tepal (removed). Note the abaxial side of the flower. **Figs. 5.26-32**. Floral development in **Floydia praelta. 26.** Polar view of left flower of a pair after initiation of the tepals (removed) and the stamen primordia. **27**. Polar view of flower pair showing the enlargement of the floral apex during the inception of the terminal carpel. **28**. Polar view of right flower of pair and oblique of left flower. **Figs. 5.29-32.** Variability of carpel orientations between a lateral orientation (Figs. 5.29-30) and diagonal orientation (Figs. 5.31-32). **29**. Polar view of right flower of pair during the early formation of the cleft on the terminal carpel. The cleft is positioned laterally in relation to the flower. **30.** Polar view of right flower after marginal expansion of the carpel showing the lateral orientation. **31**. Polar view of right flower, two stamens have been removed, showing the early formation of the cleft in a diagonal orientation. The cleft faces the alternitepalous site between the adaxial tepal and the lateral tepal closest to the principal axis (at top in picture). **32.** Polar view of right flower of the carpel showing the diagonal orientation. In all images the scale bar = 50 μm.



Figs. 5.21-5.32

Floydia praelta (Macadamieae-Floydiinae) (Figs. 5.26-32): The ratio value of the floral meristem prior to tepal organogenesis is approximately 1.2 (not illustrated). Tepal initiation is sequential, with the lateral pair appearing prior to the sagittal pair. Following tepal initiation, the ratio value range of the remaining meristem varies between 0.82 and 0.96 in different flowers. After stamen initiation, the ratio value of the floral apex (Fig. 5.26) in Floydia is 0.78 to 0.94. The floral apex increases uniformly in height, and the floral apex/carpel primordium ratio value ranges from 0.86 to 1.04 in different flowers (Figs. 5.27-28). There is variation in carpel orientation among flowers on an inflorescence in Floydia praelta (50 flowers examined): the variation was approximately 70% laterally oriented carpels, 30% diagonally oriented carpels (26% ad-lat - 4% ab-lat). The cleft forms in either a lateral position (Figs. 5.29-30) or a diagonal position (Figs. 5.31-32). In the earliest stages of carpel cleft development, the frontal/sagittal ratio value of the young laterally oriented carpels was between 0.88 and 0.92 (Figs. 5.29-30) and in the diagonal carpels, the ratio value ranged between 0.94 to 1.06 (Figs. 5.31-32).

The lateral orientation of the carpel from centric growth of the floral meristem was observed in other taxa of Knightiinae including Eucarpha deplanchii, Knightia excelsa; other genera included Lomatia fraxinifolia (Lomatiinae:Embothrieae), Opisthiolepis heterophylla (Buckinghamiinae: Embothrieae).

Lateral inception (Figs. 5.33-42) - Stenocarpus salignus (Embothrieae-Stenocarpiinae) (Figs. 5.33-37) - From the axil of each common bract, a bisymmetrical, oblate short-shoot meristem is initiated. From the lateral flanks of each short-shoot meristem, two floral bract primordia are initiated (FB in Fig. 5.33). Subtended by each floral bract, a laterally oblate floral meristem is initiated (Fig. 5.33). These meristems are bisymmetrical with ratio values of 1.39-1.50 before tepals are initiated. The lateral tepals are initiated before the sagittal tepals (Fig. 5.34). After tepal initiation (Fig. 5.34), the remaining floral meristem has a ratio value of 0.85 to 0.78 before the stamens are initiated.

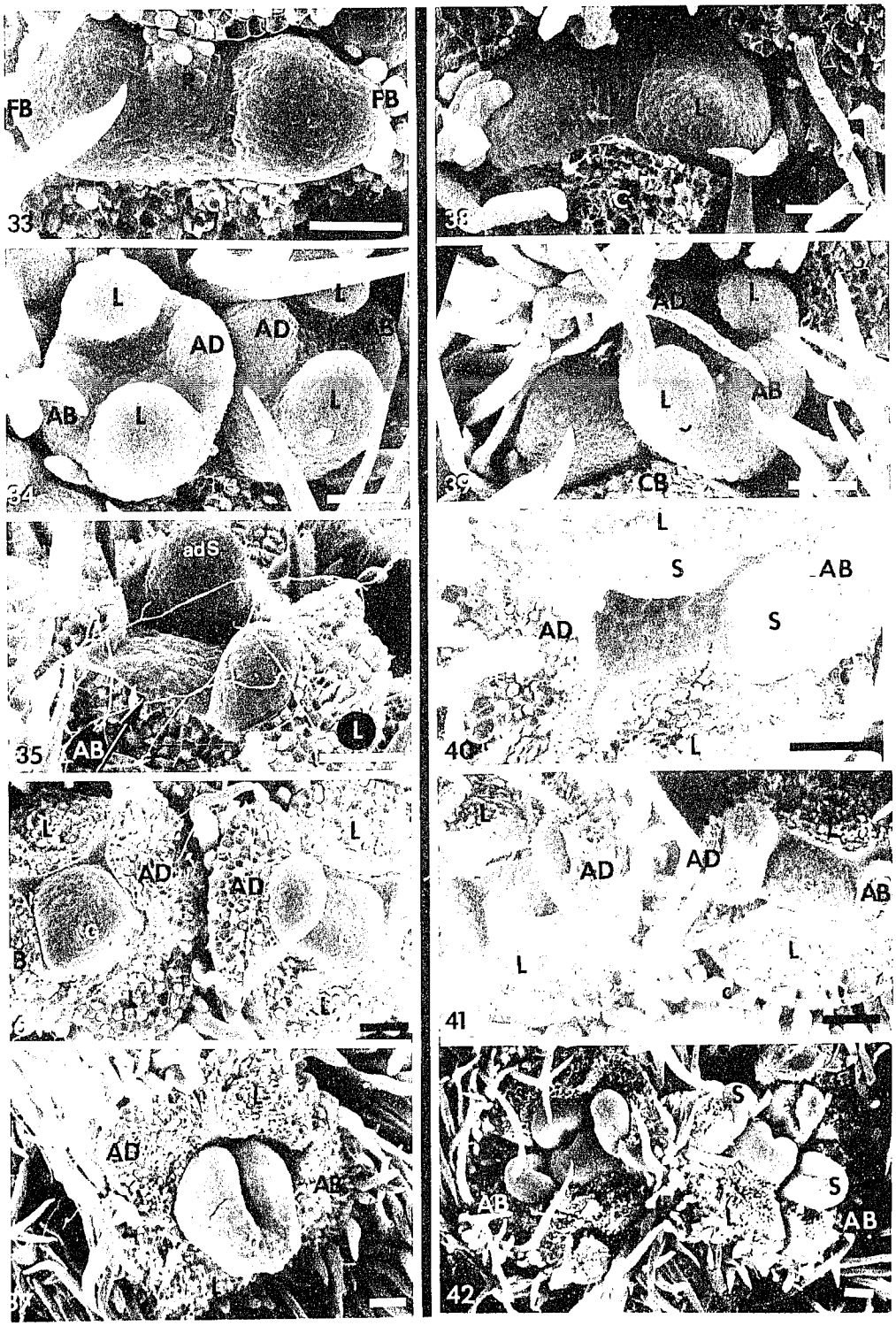
After stamen initiation (Fig. 5.35), the remaining floral meristems have a ratio value of 0.63 -0.83. The remaining floral apex has carpel inception on the common-bract side of the flower (Fig. 5.36). The ratio value of the carpel primordium is approximately 1.18. The carpel primordium heightens to about 60 μm before cleft formation. When the cleft forms, it extends ventrally to the carpel base and does not go over the summit (Fig. 5.37).

Buckinghamia celsissima (Embothrieae - Buckinghamiinae) (Figs. 5.38-42) - From a laterally oblate short-shoot meristem in the axil of a common bract, two floral meristems are initiated (Fig. 5.38). The floral meristem is bisymmetrical with a ratio value of approximately 1.2 to 1.42 just before tepal initiation. The lateral tepals are initiated first followed by the sagittals (Fig. 5.39). After tepal initiation, the ratio value of the remaining floral meristem is approximately 0.72-0.83. After stamen initiation (Fig. 5.40), the ratio value of the remaining floral meristem is 0.71 to 0.80. On the remaining floral apex, a carpel is initiated laterally, on the common bract side of each flower (Fig. 5.41); apical residuum remains on the other side. When the dorsal side of the primordium is approximately 50 μm high, the cleft begins to form on the ventral side facing the primary axis (Fig. 5.42). The ratio value of the carpel before and during carpel cleft development ranges between 0.74 before the cleft and 0.8 when the cleft is present.

Ventral-dorsal orientation (Fig. 5.4D) - The orientation of the carpel with the cleft facing the abaxial tepal (Fig. 5.4D) was observed in species of five genera, Cardwellia sublimis, Sleumerodendron austro-caledonicum, Xylomelum salicinum, Kermadecia and Bleasdalea bleasdalei (syn. Turrillia). Carpel initiateion and development of the monotypic Cardwellia sublimis is illustrated.

Cardwellia sublimis (Knightieae - Cardwelliinae) (Figs. 5.43-51) - From the inflorescence apex flanks, laterally oblate short-shoot meristems are initiated in the axils of

Figs. 5.33-42. Laterally oriented carpels among Grevilleoideae with ***lateral inception*** of the carpel. In all images except 35, the common bract of the flower pairs is at the bottom. The median sagittal plane of each flower is sideways to the length of the plate (see 3D-E for reference). **Figs. 5.33-37.** Floral development in **Stenocarpus salignus**. **33.** Polar view of short-shoot and early initiation of two floral bracts (FB) and floral meristems (M), one on each side of the short-shoot. **34.** Polar view of left flower in pair after the tepals (adT/abT/L) have been initiated. **35.** Abaxial and slightly oblique view of left flower, the floral bract scar is at the bottom of the image. The stamens have been initiated, the lateral stamen closest to the common bract and the adaxial stamen are present. The floral meristem is beginning to enlarge. **36.** Polar view of flowers in pair showing the lateral inception of the carpel (C). The carpel increases in height on the lateral side of the flower closest to the common-bract (bottom of image) more than on the other side. **37.** Right flower of pair after marginal growth and enlargement of the carpel. The cleft extends to the base of the carpel and a small residuum persists. **Figs. 5.38-42.** Floral and carpel development in **Buckinghamia celsissima**. **38.** Polar view two floral meristems, one on each side of the short-shoot. **39.** Polar view of right flower in pair after the tepals have been initiated. **40.** Polar view of right flower of pair showing the sagittally broad floral meristem after stamen (S) intitiation. The abaxial side of the flower is to the right in the image. **41.** Polar view of flowers in pair showing the lateral inception of the carpel (C) on the lateral side of floral apex closest to the common-bract (bottom of image) more than on the other side. **42.** Polar view of floral pair after marginal expansion and enlargement of the carpels. **Scale bars** = 50μm.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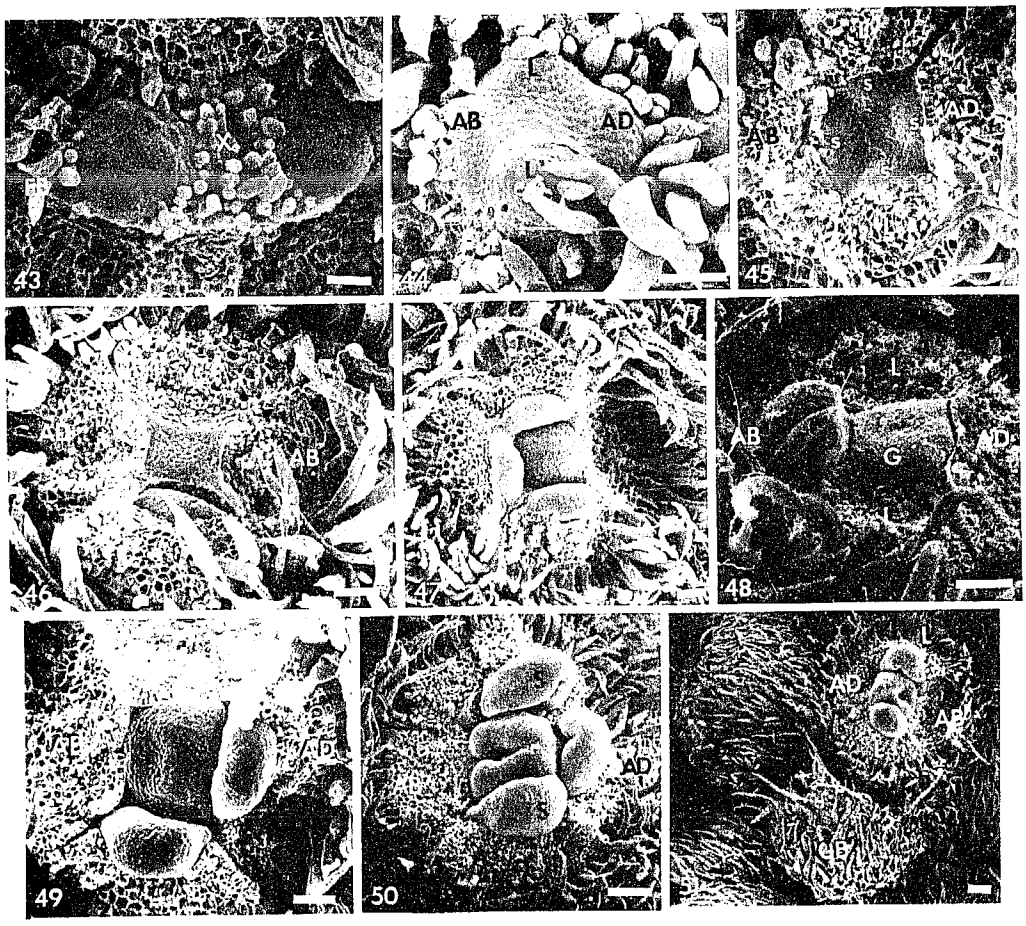
Figs. 5.33-5.42

Figs. 5.43- 51. Ventral-dorsal orientation of carpel in Cardwellia sublimis. **43**. Polar view of short-shoot and early initiation of two floral bracts (FB) and floral meristems, one on each side of the short-shoot. **44.** The left flower of a pair showing simultaneous tepal initiation. **45**. Polar view of left flower after stamen initiation. **46.** Polar view of right flower; all of the tepals and stamens have been removed and the floral meristem has begun to expand. **47.** Polar view of left flower of pair showing the terminal enlargement of the floral meristem/carpel primordium. The shape of the meristem is trapezoidal; the longer of the parallel sides is on the adaxial side. **48.** Slightly lateral view of carpel inception in left flower of a pair. The trapezoidal outline is more pronounced and the cleft is forming on the summit towards the abaxial side of the flower. **49.** Polar view of left flower in a pair as the young carpel expands. The cleft is positionally ventral-dorsal, facing the abaxial tepal of the flower (removed, scar labelled AB). **50.** Oblique abaxial view of marginal expansion and enlargement of the carpel. The cleft extends to the base of the carpel. **51.** Oblique polar view of flower pair in relation to the common bract (CB). The tepals and the sagittal stamens of the right flower have been removed, showing the ventral-dorsal orientation of the carpel. **Scale bars** = 50 μm.



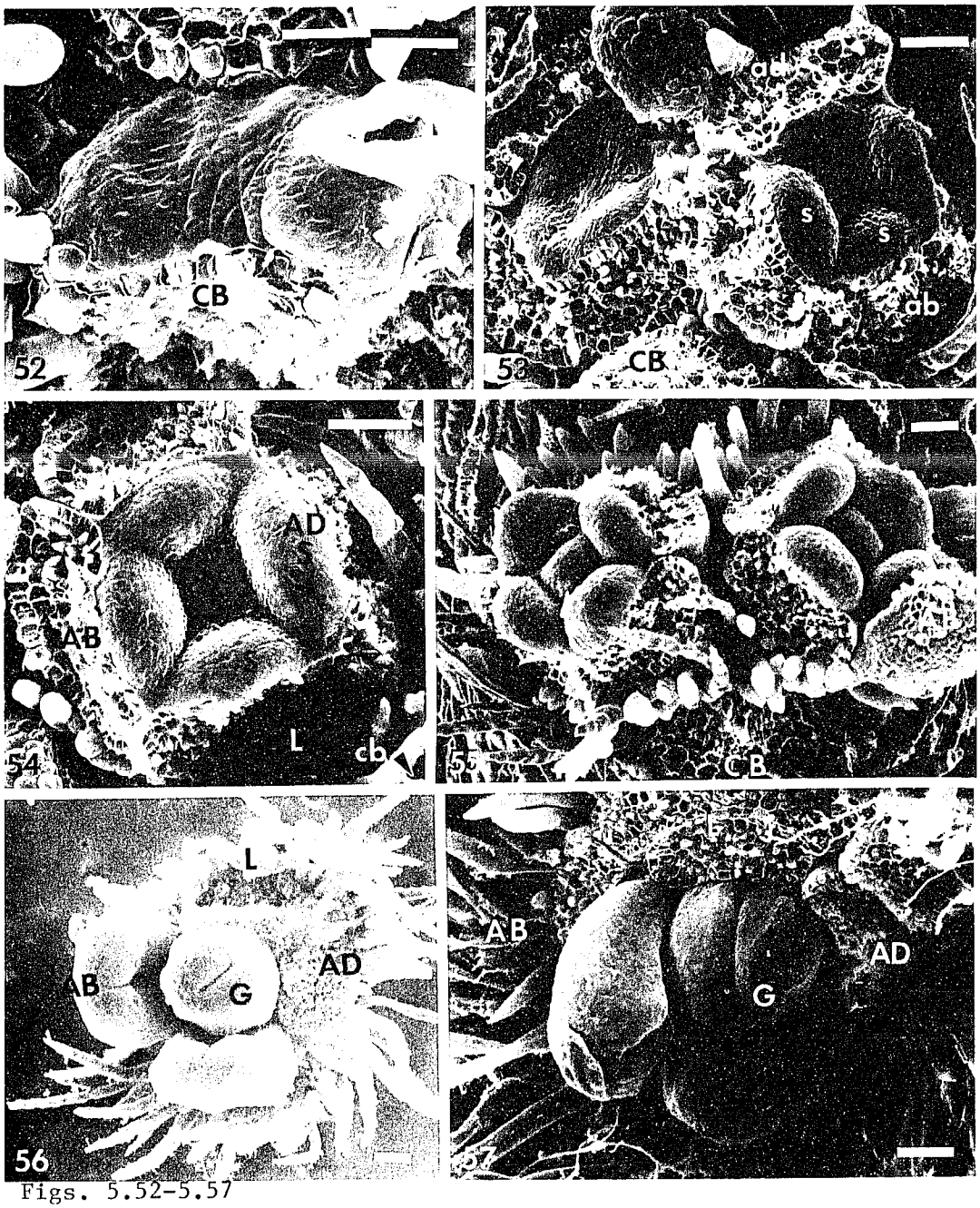
Figs. 5.43-5.51

common bracts. From each short-shoot meristem, two floral meristems are initiated (Fig. 5.43) that each have an approximate ratio value of 1.04-1.2. Tepal initiation appears simultaneous in Cardwellia (Fig.44). After tepal initiation, the floral meristem ratio value is 0.76-0.90. After stamen initiation (Fig. 5.45), the ratio value of the floral apex is 0.90-0.98. As the floral apex enlarges (Fig. 5.46), the adaxial side of the floral apex becomes wider than the abaxial (Figs. 5.47-48). A carpel is initiated at an apex height of approximately 35 μm. The carpel enlarges and forms a cleft abaxially (Fig. 5.49, 51). The cleft extends to the carpel base (Fig. 5.50). At the time of cleft formation, the ratio value of the carpel primordium is approximately 1.09 (measurements taken from the center of the flower). The ratio value of the early carpel after cleft formation is 1.2.

Diagonal Orientations (Fig. 5.4E-G) - Adaxial lateral type (Figs. 5.4E; 52-70) - The type in which the cleft of the carpel faces the alternitepalous site between the adaxial and lateral tepal closest to the primary inflorescence axis (Fig. 5.4E) appears to be the most common diagonal condition among Grevilleoideae. This type of orientation is found in Musgraveinae, Grevillea, Hakea, Embothrium, Oreocallis, Alloxylon, Telopea and different genera of Macadamieae. There are two types of enlargement of the floral apex after stamen initiation: **terminal inception** in which the floral apex converts completely to a carpel and **lateral inception** in which the carpel is initiated laterally from the floral apex.

Terminal inception (Figs. 5.52-57) - Macadamia integrifolia - (Macadamieae-Macadamiinae) - The floral meristem prior to tepal organogenesis (Fig. 5.52) is bisymmetrical with a ratio value of approximately 1.2. Tepal and stamen initiation were not observed. After stamen initiation (Figs. 5.53-54) the relative ratio value of the floral apex varies between 0.97 (Fig. 5.53) and 0.85 (Fig. 5.54) in different flowers . The floral apex is converted completely into the carpel (Fig. 5.55) and the floral apex/carpel primordium ratio value ranges from 0.89 to 1.06. There is variation in carpel orientation

Figs. 5.52-57. Adaxial-lateral orientation of carpel and ***terminal inception*** in Macadamia integrifolia. **52.** Oblique polar view of short-shoot and early initiation of two floral meristems, one on each side of the short-shoot. **53.** Polar view of flower pair after stamen initiation; note that the flowers are slightly skewed, and the abaxial tepal scars are skewed downward slightly. **54.** Polar view of left flower of a pair after stamen initiation. The frontal and sagittal diameters are ~ equal. **55.** Polar view of floral pair as the floral meristem enlarges. **56.** Polar view of left flower showing the cleft facing the alternitepalous site between the adaxial and lateral tepal closest to the common bract. **57.** Frontally oblique view of right flower of a pair showing the lateral carpel orientation, present in some flowers of this taxon. **Scale bars** = 50 μm.

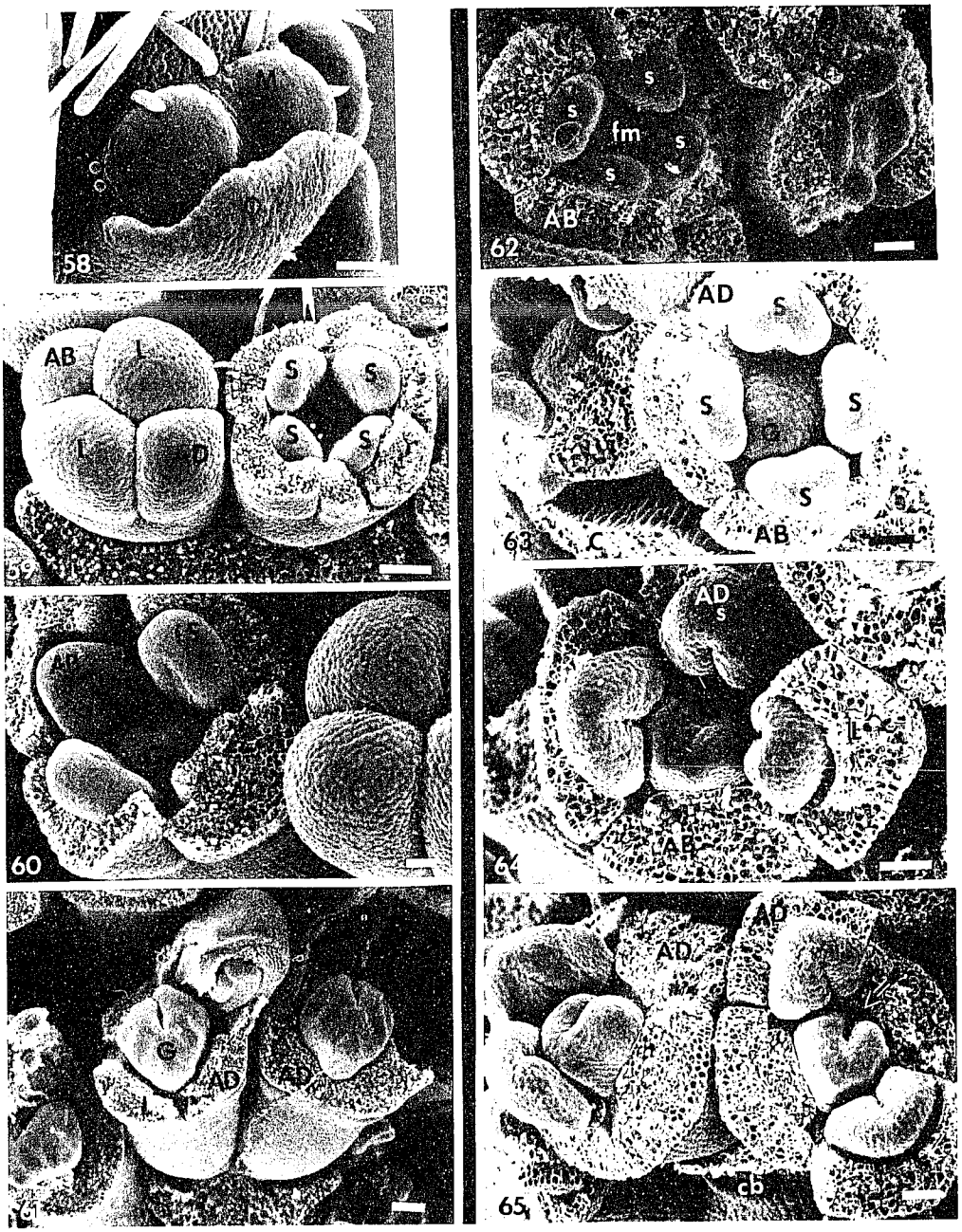


Figs. 5.52-5.57

among flowers in each inflorescence of M. integrifolia and M. tetraphylla. Variation was consistent in six different collections of Macadamia integrifolia and three different collections of M. tetraphylla examined (50 flowers on 5 different inflorescences in each collection). Approximately 60% of the flowers of both species had diagonally oriented carpels (Fig. 5.56) and 40% had laterally oriented carpels (Fig. 5.57). In the earliest stages of carpel cleft development, the ratio value of the young diagonally oriented carpels was between 0.95 and 1.1 and in the laterally oriented carpels the ratio values ranged from 0.89 and 0.93.

Lateral inception (Figs. 5.58-75) - Telopea mongaensis (Figs. 5.58-61) - In the axil of each common bract along glabrous primary inflorescence axes, a bisymmetrical, oblate short-shoot meristem is initiated. From the lateral flanks of each short-shoot meristem, two floral meristems are initiated (M, Fig. 5.58). Each floral meristem has a ratio value of 0.86-1.2 before tepals are initiated. The size of the floral meristem and ratio values are difficult to measure in this taxon due to compression of the floral meristems; tepal initiation is also difficult to determine and variable. They can be initiated simultaneously as a whorl, in pairs with the sagittals appearing first, or in a unidirectional sequence from the abaxial side (not illustrated; see Chapter 4). After tepal and stamen initiation (Fig. 5.59), the remaining floral apex has a ratio value of 1.0. The carpel is initiated on one corner of the floral meristem, and increases in height (Fig. 5.60) forming a 'crescent-shaped' or 'U-shaped' carpel primordium. The concave portion of the carpel primordium is the cleft or the ventral side. The carpel primordium increases in height and the cleft faces between the abaxial and lateral tepal closest to the primary inflorescence axis (Fig. 5.61). The orientation in Telopea mongaensis is peculiar compared to the other species and genera of Embothriinae; in the other species and genera of Embothriinae, the carpel cleft faces the alternitepalous site between the adaxial and lateral tepal margins.

Figs. 5.58-65. Diagonal orientation of carpel and ***lateral inception***. **Figs. 5.58-61.** Floral initiation and carpel initiation in **Telopea mongaensis**. **58.** Oblique polar view of short-shoot and early initiation of two floral meristems (M), one on each side of the short-shoot. **59.** Polar view of flower pair showing sagittally-incomplete aestivation pattern in left flower and the floral meristem after stamen initiation in the right flower. **60.** Oblique adaxial view of carpel inception laterally. The early carpel primordium is 'U-shaped.' **61.** Polar view of flower pair showing orientations of carpels after carpel enlargement. **Figs. 5.62-65.** Carpel initiation in **Embothrium coccineum**. **62.** Polar view of left flower of pair after stamen initiation, showing the flat floral meristem. **63.** Polar view of right flower of pair showing the lateral inception of the carpel and the alternitepalous position of the carpel. **64.** Later stage of carpel development showing cleft formation on the 'U-shaped' prmordium. **65.** Polar view of flower pair after carpel enlargement showing the orientation of the carpels, the cleft facing the alternitepalous site between the adaxial and lateral tepal closest to the principal axis. An apical residuum persists ventral to the carpel (arrow). **Scale bars** = 50 μm.

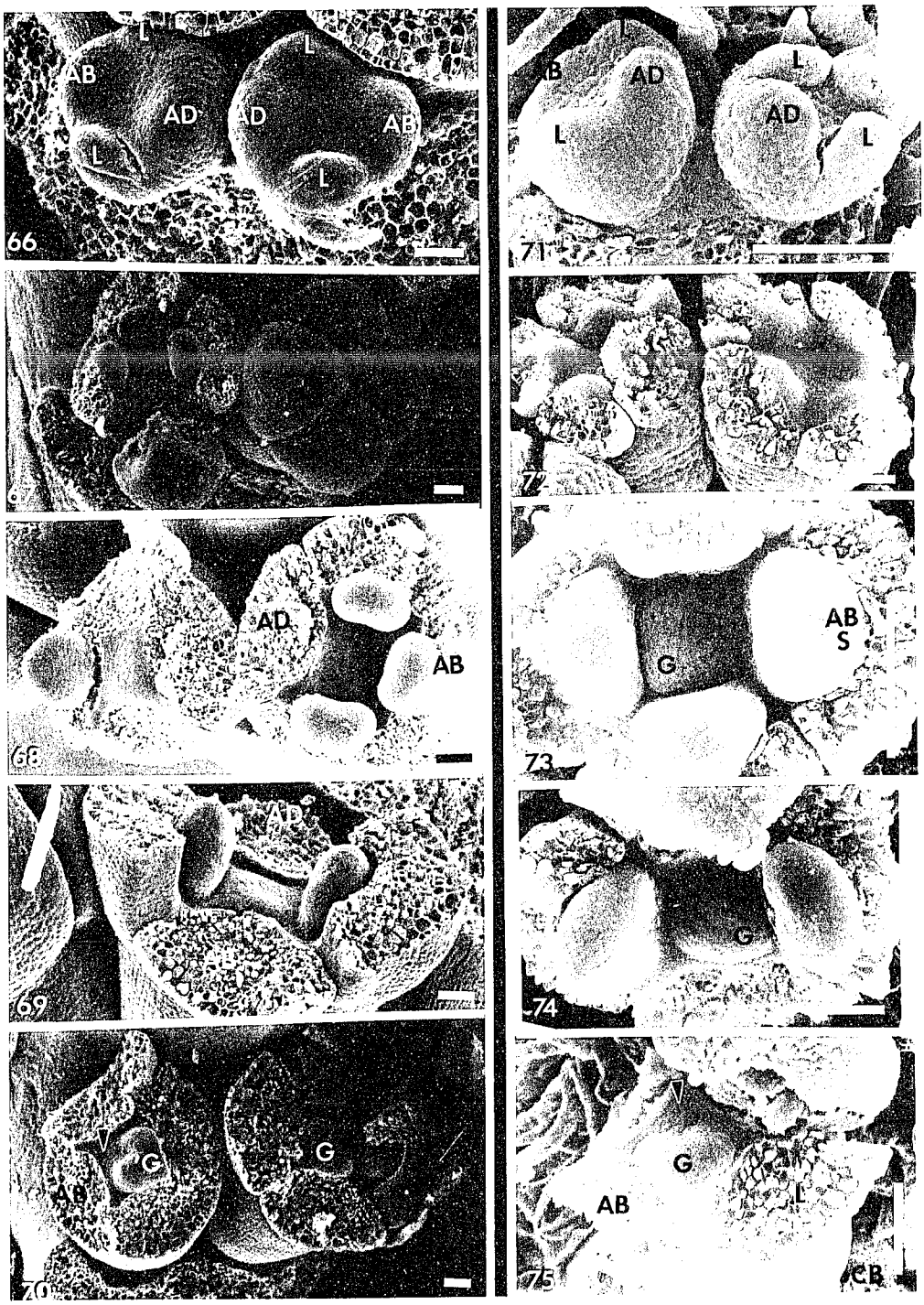


Figs. 5.58-5.65

Embothrium coccineum (Figs. 5.62-65) - From the axil of each common bract along a glabrous terminal inflorescence, a short-shoot meristem is initiated, which then initiates two flower meristems. After stamen initiation (Fig. 5.62), the ratio value of the floral meristem differs on different flowers. In most flowers (90% of 40 examined on 15 inflorescences), the ratio value is between 0.95 and 1.02. In the other flowers, the ratio value is between 0.89 and 0.94. The remaining floral meristem initiates a carpel laterally, proximal to the common bract (Fig. 5.63). At a carpel height of approximately 50 μm, the cleft forms on the sloping ventral side of the carpel primordium (Fig. 5.64). The carpel primordium enlarges and the cleft extends to the carpel base (Fig. 5.65). There is a portion of the floral meristem/receptacle next to the ventral side of the carpel that does not get used in the initiation of the carpel (arrow in Fig. 5.65): a nectariferous gland will be initiated on this residuum at a much later stage of development (not illustrated). In some flowers (approximately 6% of 30 examined), the cleft forms facing a lateral tepal (not illustrated); such flowers were generally found in the upper two nodes of each inflorescence.

Grevillea wilsonii (Figs. 5.66-70) - Two floral apices (Fig. 5.66) are initiated from the short-shoot meristem in a common-bract axil. Tepal initiation appears simultaneous. After tepal initiation, the ratio value of the floral meristem is approximately 0.95. After stamen initiation (Fig. 5.67), the floral meristem ratio value is approximately 1.0. The carpel is initiated laterally on the floral meristem in the corner of the flower closest to the common bract (Figs. 5.68-69). The carpel primordium is initiated laterally and becomes 'U-shaped' (Figs. 5.68-70). The portion of the floral meristem/receptacle next to the ventral side of the carpel is not incorporated in the development of the carpel; a nectariferous gland develops on this residuum at a later stage in development (not illustrated).

Figs. 5.66-75. Diagonal orientations and ***lateral inception*** in species of Grevilleae. **Figs. 5.66-70**. Abaxial-lateral initiation and orientation of the carpel in **G. wilsonii**. **66**. Polar view of flowers on short-shoot after tepal initiation. **67**. Polar view of flowers in pair. The tepals and the lateral stamen closest to the common bract have been removed in the left flower showing the floral meristem prior to carpel inception. The sagittally-incomplete aestivation pattern is shown in the right flower. **68.** Polar view of right flower in pair showing the lateral inception of the carpel in an alternitepalous (really alternistamenous) between the abaxial and lateral tepal closest to the common bract. The early carpel primordium is being initiated in the corner (the dorsal side). **69.** Frontal view of right flower in pair showing the lateral position of the 'U-shaped' form and carpel. **70**. Polar view of pair after the carpel has enlarged showing a residuum (arrow head) and the orientation of the cleft facing the alternitepalous site between the abaxial and lateral tepal closest to the common bract. **Figs. 5.71-75**. Floral development and carpel initiation in **G. vestita**. **71.** Polar view of flowers on short-shoot after tepal initiation. **72.** Polar view of flowers in pair after initiation of the stamens. All tepals have been removed. **73.** Polar view of left flower of pair showing the lateral inception of the carpel primordium in the alternitepalous site between the abaxial and lateral tepal closest to the common bract (an opposing position to that described for G. wilsonii, compare to left flower in 63). **74**. Adaxial polar view of early carpel formation showing the corner initiation compared to the apical residuum. **75**. Polar view of left flower in pair showing orientation of cleft facing the alternitepalous site between the adaxial and lateral tepal. An apical residuum persists (arrow-head). **Scale bars** = 50 μm.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Figs. 5.66-5.75

Grevillea vestita (Figs. 5.71-75) - Two floral primordia (Fig. 5.71) are initiated from the short-shoot meristem. The ratio value of the floral apices prior to tepal initiation is 1.04. Tepal initiation appears simultaneous. After tepal initiation, the ratio value of the floral meristem is approximately 0.98. After stamen initiation (Fig. 5.72), the floral meristem ratio value is approximately 1.0. The carpel is initiated (Fig. 5.73) in the corner of the floral apex facing the common bract (Fig. 5.73-74). The ratio values on different flowers range between 1.01 and 0.97. The carpel primordium is initiated laterally and becomes 'U-shaped' (Figs. 5.75). The cleft will form on the vental side of the carpel primordium (Fig. 5.75). The portion of the floral meristem/receptacle next to the ventral side of the carpel does not get incorporated in the development of the carpel; a nectariferous gland develops at a later stage in development (not illustrated).

Similar patterns of lateral carpel inception with adaxial-lateral cleft orientations have been observed in G. glabella, G. baileyana, G. asplenifolia, G. lavandulacea and Hakea myrtoides. Payer (1857) described a similar pattern of initiation in G. thelemanniana as did Baum (1952).

Abaxial lateral type (Figs. 5.4F; 76-83) - The arrangement in which the cleft faces an alternitepalous site between the abaxial and lateral tepal closest to the primary inflorescence axis (Fig. 5.4F) is common among members of Banksiinae. Floral initiation of Banksia pulchella is described.

Two floral apices are initiated in floral bract axils (Fig. 5.76) from the short-shoot meristem. Tepals are initiated sequentially; the lateral tepals are initiated (left flower in 76), followed by the initiation of the tepals in the sagittal positions (Fig. 5.77). The floral meristem prior to tepal initiation is 90 μm x 80 μm with a ratio value of 1.13. After tepal initiation, the ratio value is approximately 1.0. The stamens are initiated virtually simultaneously (Fig. 5.78) and the ratio of the meristem is approximately 1.04. The floral

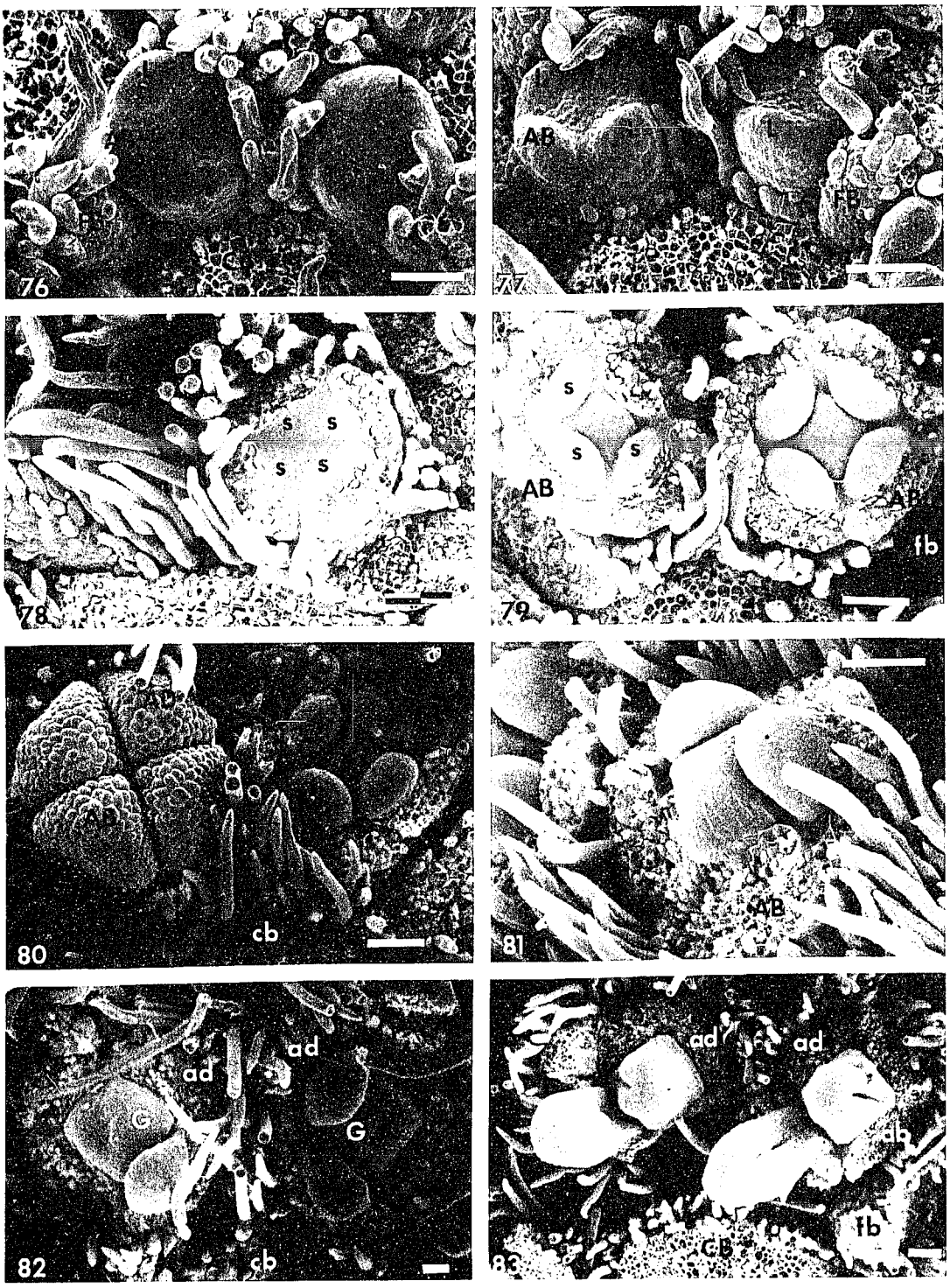
meristem enlarges uniformly (left flower in Fig. 5.79) and as it enlarges, it develops 'corners' alternate with the stamens (right flower in Figs. 5.79, 80). The ratio value of the meristem, as it enlarges is approximately 1.02, and as the 'corners' develop, the ratio value approaches 1.0. The floral apex becomes almost quadrangular (Figs. 5.79-81) at a height of approximately 45 μm. At a height of approximately 50 μm, there is an expansion of the floral apex/carpel primordium (Figs. 5.79-81): the meristem enlarges in opposite corners becoming slightly diamond-shaped and the the cleft becomes evident in a plane perpendicular to the longer side of the floral meristem. The dorsal side of the carpel primordium begins to heighten (Fig. 5.82). The ratio value of the carpel primordium is approximately 1.0. Carpel height increases and the cleft extends ventrally but not to the base of the carpel (Fig. 5.83). All of the floral apex appears to be converted in the terminal carpel.

Similar patterns of terminal carpel inception and the orientation of the cleft facing the alternitepalous site between the abaxial and lateral tepal were observed in B. praemorsa, B. ericifolia and Dryandra kippistiana. Similar orientations of the carpels were observed in B. menziesii and B. coccinea by Fuss and Sedgley (1991).

Distal abaxial type (Figs. 5.4G; 84-89) - The arrangement in which the cleft faces the alternitepalous site between the abaxial and distal lateral tepal closest to the common bract (Fig. 5.4G) was observed in only two species of Lambertia. L. inermis is described and illustrated.

Lambertia inermis (Macadamieae; Lambertiinae) - In the axils of each of three cuneate common bracts, a bisymmetrical oblate short-shoot meristem is initiated. As each meristem enlarges, two floral bract primordia are initiated on the lateral, proximal flanks of the meristem (arrows, Fig. 5.84). The floral bracts become suppressed following initiation (arrow heads in Fig. 5.85). Concomitantly, two floral meristems are initiated

Figs. 5.76-83. Abaxial-lateral type orientation. Floral development and carpel initiation in Banksia pulchella. **76.** Polar view of floral meristems on short shoot. In the axils of floral bracts (FB) the lateral tepals are initiated prior to (right flower) the sagittal tepals. **77.** Oblique polar view of flowers in pair showing all four tepals. **78.** Polar view of right flower in pair. The tepals have been rmoved and the stamen primordia have been initiated. **79.** Polar view of floral pair showing the enlargement of the floral meristem after stamen initiation. In the left flower, the meristem has not enlarged as much as the right flower. **80.** Polar view of floral pari showing sagittally-incomplete aestivation pattern of the tepals in the left flower and the expansion of floral meristem in the right flower. **81.** Abaxial view of right flower in pair showing the carpel primordium. **82.** Polar view of carpel cleft formation in both flowers of a pair. The dorsal side of the carpel is higher than the ventral side. **83.** Polar view of carpel enlargement in both flowers of a pair. The carpel cleft faces the aleternitepalous site between the abaxial and lateral tepal closest to the principal axis. The carpel cleft does not extend to the carpel base. **Scale bars** = 50 μm.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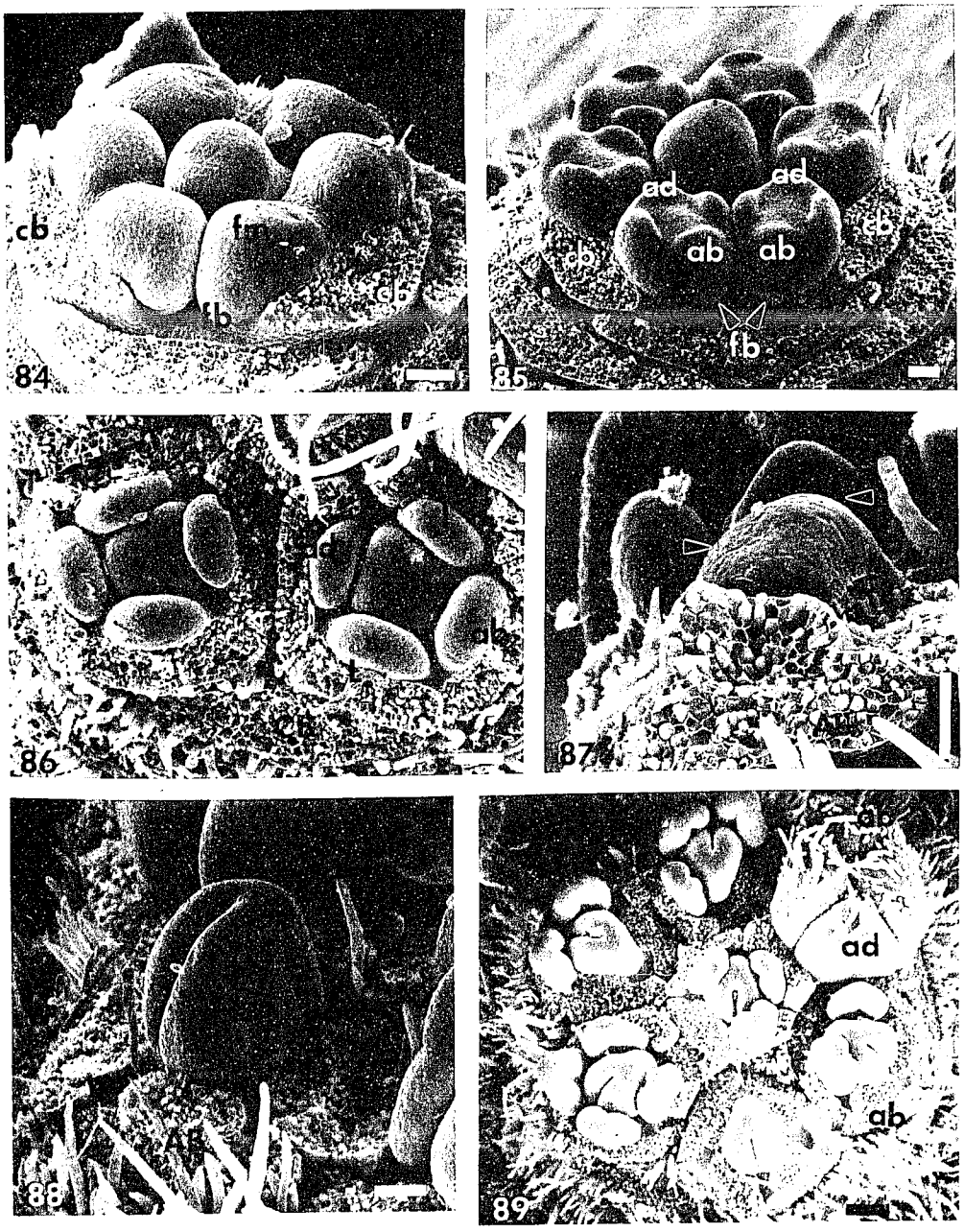
Figs. 5.76-5.83

Figs. 5.84-89. Distal-abaxial carpel orientation and flower development in Lambertia inermis. **84**. Oblique view of capitate inflorescence and the initiation of floral meristems on three short-shoot meristems. On the lateral flanks of each short-shoot, a floral bract is initiated (FB). **85**. Oblique polar view of flowers on capitate inflorescence after the initiation of the tepals. The floral bracts (arrows) are suppressed and trichomes are differentiating in these sites. **86**. The enlargement of the early carpel primordium forces the stamens to separate slightly. **87.** Abaxial view of carpel primordium showing the unequal height between the dorsal side (arrow head on right) and the ventral side (arrow head on left). **88.** Oblique abaxial view of carpel after cleft formation. **89.** Polar view of capitate inflorescence showing the orientation of the carpels is distal-abaxial, the carpel cleft facing the alternitepalous site between the abaxial tepal and lateral tepal closest to the common bract. **Scale bars** = 50 μm except in 89=100μm.



Figs. 5.84-5.89

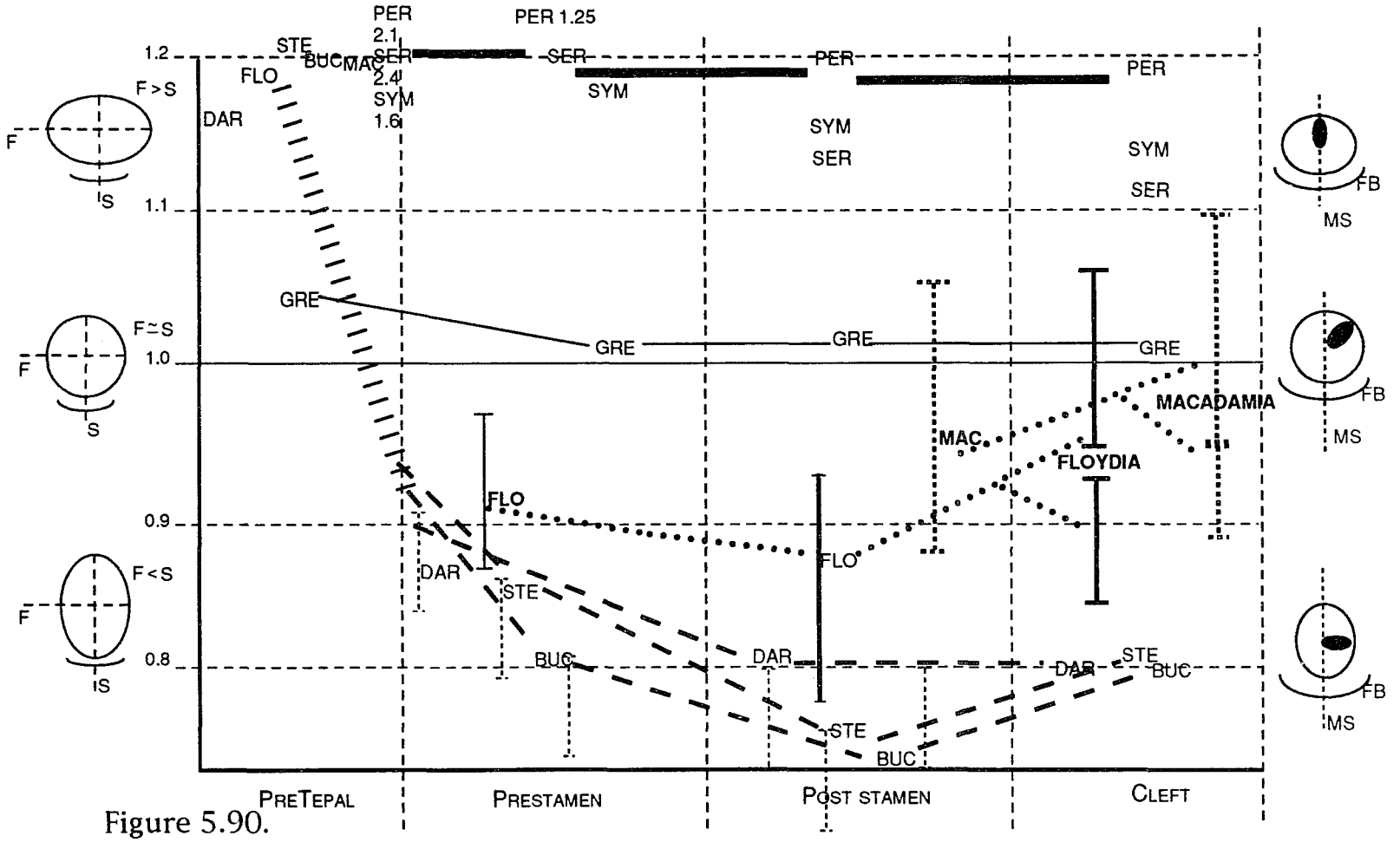
from each short-shoot meristem (Fig. 5.84). Tepals appear to be initiated almost simultaneously although the tepals in the lateral plane are slightly larger than the adaxial and abaxial tepals (Fig. 5.85). Stamens are initiated simultaneously (not illustrated). The remaining floral apex broadens uniformly at first. Then a carpel is initiated on the portion of the apex, furthest away from the common bract (arrow heads, Fig. 5.87). There is differential enlargement in the 'corners' of the floral meristem alternating with the stamen primordia (Fig. 5.86) that continues in two opposing corners (arrows in Fig. 5.86) more than the other corners, resulting in a diamond-shaped carpel primordium. The cleft forms on the ventral sloping side of the carpel primordium (Fig. 5.88) and faces the point of intersection between the abaxial and lateral tepal closest to the common bract (Fig. 5.89).

Meristem shapes and relative sizes - The ratio values of the floral meristem and early carpel of selected proteaceous taxa are summarized in Figure 5.90. In taxa of Persoonioideae and Proteoideae with dorsiventral carpel orientations, the frontal diameter of the floral meristem through organogenesis is greater than the sagittal diameter (frontally bisymmetrical shape; values above 1.0). Taxa with diagonal carpel orientations have ratio values between 1.1 and 0.90 (roughly actinomorphic shape). Taxa with lateral orientations have ratio values that are generally less than 0.90 (sagittally bisymmetrical shape) at the time of carpel initiation.

DISCUSSION

This comparative ontogenetic study of carpel initiation and floral organization is discussed under four topics. First of all, it defines variability in floral organization of Grevilleoideae and indicates conserved taxonomic levels. Secondly, it provides insights into the developmental processes responsible for diverse carpel orientations. Thirdly, the ontogenetic diversity involved with carpel initiation is discussed in terms of evolutionary, structural and selective constraints involved in floral organization. Fourthly,

Fig. 5.90. Meristem shapes and relative sizes. Graph form illustration of ratio values of the frontal diameter/sagittal diameter in selected taxa. The columns of the graph represent discreet developmental events through developmental time. In all case there is variation present during the interim between these stages. The values are included as there is a certain ambiguity in comparing similar stages in developmental studies. In all cases except Floydia and Macadamia the point of the graph of each taxon represents the upper range. In taxa of Persoonioideae and Proteoideae with dorsiventral carpel orientations, the frontal diameter of the floral meristem through organogenesis is greater than the sagittal diameter (bisymmetrical shape, frontally broader than sagittally; values above 1.0). Taxa with diagonal carpel orientations have ratio values between 1.1 and 0.90 (roughly actinomorphic shape). Taxa with lateral orientations have ratio values that are generally less than 0.90 (bisymmetrical shape, sagittally broad) at the time of carpel initiation. Abbreviations: STE=Stenocarpus salignus; FLO=Floydia praelta; BUC=Buckinghamia celsissima; DAR=Darlingia darlingiana; PER = Persoonia myrtilloides; SER = Serruria pedunculata; SYM=Symphionema montana; GRE= Grevillea vestita; MAC=Macadamia integrifolia.


PER
2.1
SER
2.4
SYM
1.6
PER 1.25
SER
SYM
PER
SYM
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1.2
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PRETEPAL
PRESTAMEN
POST STAMEN
CLEFT

Figure 5.90.

developmental syndromes responsible for certain carpel orientations are associated with systematics; such associations could provide evidence supporting phylogenetic relationships.

Carpel orientations among Grevilleoideae - How many orientations are there among Grevilleoideae? - The orientation of the carpel among genera of Grevilleoideae is quite variable. Given that there are eight possible orientations in a four-merous proteaceous flower, six of the eight were found (Fig. 5.4). The only two carpel orientations lacking among Grevilleoideae examined are the diagonal where the cleft faces the alternitepalous site between the adaxial and lateral tepal closest to the common bract, and the orientation where the cleft faces the lateral tepal closest to the common bract. It should be noted that Venkata Rao (1971) suggested the former in one species of Orites and one species of Helicia; neither of these two taxa were available for investigation. Such variability in carpel orientations among other single-carpellate angiosperms has never been reported and as far as I can tell is unique to Grevilleoideae.

Are the carpel orientations generically conserved? - In general, the type of carpel orientation is specifically and generically conserved. However, in certain taxa the carpel orientation is variable at either the intraspecific level, intrageneric level or inter-generic level.

Intraspecific variability - In several species, the orientation of the carpel varied between a diagonal, lateral and/or a dorsiventral orientation. Taxa where carpel orientation varied between a lateral and diagonal condition include Macadamia integrifolia (60% diagonal), M. tetraphylla (75% diagonal), Floydia praelta (70% lateral) and some flowers of Cennarhenes nitida (Proteoideae: Cennarheniinae). Diagonal orientations in some flowers of Cennarhenes were reported by Venkata Rao (1961). Variability between

diagonal and dorsiventral orientations was observed in Athertonia diversifolia (60% dorsiventral; three inflorescences examined). An interesting variable carpel orientation was observed in Stirlingia latifolia (Douglas, chapter 3). In Stirlingia, the orientation of the carpel varied between a dorsiventral and a lateral orientation. In different inflorescences, all flowers were either lateral or dorsiventral; the variation in carpel orientations appears to be plant-specific. A more thorough sampling of the population is necessary prior to any conclusions on this example of variability (Douglas, chapter 3).

Intrageneric variability - Among species of Grevillea the orientation of the carpel varied. In most species examined, the carpel cleft was diagonal and faced the alternitepalous site between the adaxial and lateral tepal. In G. wilsonii the carpel cleft faced the alternitepalous site between the abaxial and lateral tepal. No other examples of intrageneric variability were observed for this character.

Intergeneric variability - Among genera in Embothriinae (sensu Johnson and Briggs, 1975; Crisp and Weston, 1987), the carpel cleft faces the alternitepalous site between the adaxial and lateral tepals (Fig. 5.56). In Telopea mongaensis, the cleft faces the alternitepalous site between the abaxial and lateral tepal closest to the primary inflorescence axis (Fig. 5.56). The organization of the flower in this taxon is also peculiar relative to the other taxa in the subtribe: the flowers in a pair are convexly skewed (Chapter 4).

What are the developmental events associated with different orientations? - There are differing developmental processes associated with the diverse orientations of carpels among Grevilleoideae. In most taxa, the carpel is terminal: the product of the conversion of the remaining floral apex. In other taxa the carpel appears to be initiated from a lateral position on the floral apex. Among all of the taxa, there is correlation between the shape of the floral apex and the orientation of the cleft: the ventral

Fig. 5.91. Graphic illustration depicting the developmental processes involved with carpel orientations among Proteaceous taxa investigated. The taxa are divided into two groups based on the position of the carpel at initiation. Letters correspond to orientation and developmental type found in the taxa described and taxa investigated but not described in Table 5.1.

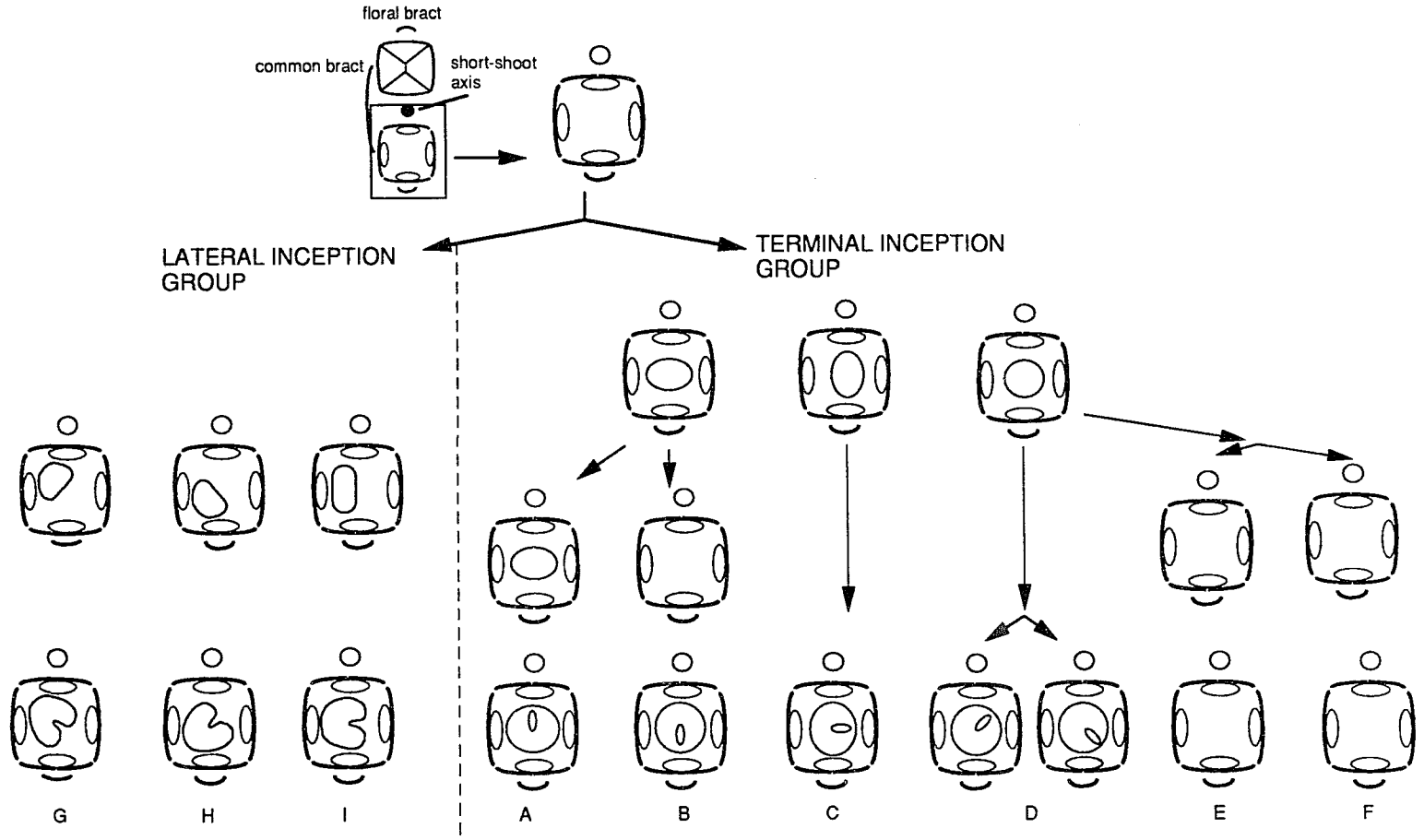

floral bract
common bract
short-shoot axis
LATERAL INCEPTION GROUP
TERMINAL INCEPTION GROUP
G
H
I
A
B
C
D
E
F

Figure 5.91.

side of the carpel forms in a plane perpendicular to the longest axis of the floral apex. Among some taxa, there is anomalous enlargement and expansion of the floral apex/carpel primordium that occurs prior to the formation of the cleft. Figure 5.91 is a diagrammatic representation of the different processes involved with carpel organogenesis and early carpel morphogenesis among Proteaceous taxa.

Carpel organogenesis - The enlargement of the floral apex after stamen initiation and before carpel initiation, prior to the development of the cleft, and the subsequent position of the carpel varies in two ways: the carpel is either terminal (terminal inception; Fig. 5.91 Group A) or lateral (lateral inception; Fig. 5.91 Group B).

Terminal carpel inception - This type predominates among most proteaceous taxa representing all subfamilies. After stamen initiation and independent of carpel orientation, the floral apex enlarges throughout and is fully converted into a carpel primordium (Terminal inception group in Fig. 5.91). The cleft will form as a dimple, usually on one side of the carpel primordium. In these taxa, developmental evidence indicates that the carpel is terminal. It appears from morphological evidence and limited anatomical evidence (Chapter 2), that the entire floral apex is utilized and converted into the carpel in these taxa. Other single-carpellate angiosperms also share this apical conversion pattern including Amygdalus communis (Brooks, 1940), Drimys winterii (Tucker, 1959), D. lanceolata (Tucker and Gifford, 1966 a,b), Pseudowintera transversii (Sampson and Kaplan, 1970); species of Lauraceae (Endress, 1972); Peperomia spp. (Tucker, 1980); Monodora crispata (Leins and Erbar, 1982); Potamogeton zosteriformis (Posluszny, 1981); species of Chloranthaceae (Endress, 1987), Acacia baileyana (Newman, 1934; Derstine and Tucker, 1991) and A. suaveolens and A. longifolia (Newman, 1936a,b). Considering that the terminal conversion of the floral apex into a carpel occurs in most proteaceous genera (all of Persoonioideae, Proteoideae,

Carnarvonioideae, and most genera of Grevilleoideae) it can be considered the general condition.

Lateral carpel inception - In the other taxa, the remaining floral meristem does not convert completely into a carpel (Lateral inception group in Fig. 5.90: Grevilleae, some Embothrieae). In these taxa, there is carpel initiation on one side or in one corner, an alternitepalous position, of the floral apex after stamen initiation. The larger or higher side is the carpel initial and will be the dorsal side of the carpel. In this case the carpel appears to be laterally inserted, and an inactive apical meristem residuum persists beside the carpel.

A long-standing controversy in flower evolution is whether the single carpel is a terminal or lateral structure (Newman, 1936a, b; Thompson, 1936 a, b; Brooks, 1940; Tucker and Gifford, 1966a). Considering the well-supported hypothesis that the carpel is a leaf homologue (Goethe, 1790), one would expect to find vestigial evidence of the floral residuum in single-carpellate taxa, particularly if the carpel is a lateral organ (Newman, 1936a,b). Apical residuum in unicarpellate flowers is rare. In most ontogenetic studies of single-carpellate taxa, the single carpel is the product of conversion of the floral apex (Tucker and Gifford, 1966 a,b; Sampson and Kaplan, 1970; Posluszny, 1981; Tucker, 1987; Derstine and Tucker, 1991; Douglas, Chapter 2).

There are two equally plausible hypotheses concerning lateral carpel inception. The first is that the single-carpellate condition among all Proteaceae is derived from a multicarpellate (bicarpellate and apocarpous) ancestor and that the lateral orientation is the result of a loss of the carpel on the other side; hence the residuum has been maintained as a plesiomorphic condition. A phylogenetic analysis of the family should permit testing of such a hypothesis. It should be noted that two-carpellate proteaceous flowers are

extremely rare although Venkata Rao (1966) reported that a couple of flowers had two carpels in species of Hakea laurina.

The second hypothesis, derived from comparative developmental and phylogenetic studies, is that the lateral inception of the carpel is a derived condition (or a reversal to a general angiospermous organization). There are several types of evidence supporting this contention. In general, lateral initiation of the carpel occurs among the hypothetically derived Grevilleoideae (Johnson and Briggs, 1975; Douglas, Chapter 6). Secondly, in all of these taxa, a very large nectariferous semi-circular disk (another derived feature) is initiated from the apical residuum/floral receptacle next to the ventral side of the carpel later in development. In addition, lateral inception does not always preclude the later involvement of the remaining floral apex in enlargement of the carpel as in Buckinghamia and Stenocarpus. In fact, there appears to be a morphogenetic grade among taxa; the floral apical residuum enlarges and becomes involved in carpel development in some taxa (less derived taxa sensu Johnson and Briggs, 1975; Buckinghamia, Stenocarpus). In the more derived taxa (sensu Johnson and Briggs, 1975) the apical residuum persists until the hippocrepiform nectary is initiated as in Grevilleae, Telopea, Embothrium. Future anatomical investigations should provide additional information concerning the fate of the floral apex and the initiation of the carpel.

Orientation of the carpel and available space - Among most of the taxa, there is a correlation between the shape of the floral apex after stamen initiation and the orientation of the carpel. The cleft forms in a plane perpendicular to the longest diameter of the enlarging floral apex (Fig. 5.85).

In most grevilleoid flowers, the cleft forms on a perpendicular axis to the longest diameter of the floral apex (Fig. 5.85). In many other apocarpous angiosperms, the cleft forms perpendicular to the longest diameter of the floral meristem/carpel primordium

(Payer, 1857; Grégoire, 1938; van Heel, 1981, 1983, 1984, for examples). Examination of the ratio values of the floral apex (Fig. 5.85) supports such a relationship. In the grevilleoid taxa with diagonally oriented clefts, the floral apex has a ratio value close to 1.0. In taxa with dorsiventrally oriented clefts, the ratio value of the floral apex is greater than 1.1 or the apex is frontally broad. In taxa with laterally oriented clefts, the ratio value is usually less than 0.9.

An interesting corollary supporting a relationship between shape of the meristem and carpel orientation comes from the taxa with varying carpel orientations. In Macadamia and Floydia (also some flowers of Embothrium not illustrated in Fig. 5.85), the carpel cleft varies between a diagonal and lateral orientation in different flowers. Ratio values of median frontal diameter/median sagittal diameter of the early carpel primordium (just after formation of the cleft) shows two discreet size ranges associated with the different orientations. Floral meristems, before carpel initiation, that have a ratio value less than 0.92 in flowers of Floydia praelta and 0.93 in Macadamia integrifolia had diagonally oriented carpels. There appears to be a relationship between size and space allocations and the orientation of the carpel in these taxa. This correlation also supports a relationship between the longest diameter of the floral apex (available space) and the orientation of the carpel.

In Cardwellia sublimis (and Turrillia bleasdalei not illustrated), after stamen initiation, the floral meristem enlarges differentially. The adaxial portion of the floral meristem is longer than the abaxial portion, and is the floral apex is thusly trapezoidal in outline. The cleft will form on the abaxial side in this case, furthest away from and perpendicular to the longest side of the pre-carpel primordium.

Polarity: If the cleft develops in the plane perpendicular to the longer diameter of the floral apex, why doesn't the cleft develop on the other side of the floral apex/carpel

primordium? In most taxa, the floral apex/carpel primordium appears bisymmetrical but its developing carpel shape makes it zygomorphic. From developmental evidence, in most taxa with a single carpel, the median sagittal plane of the carpel faces and bisects the primary inflorescence axis: in multicarpellate flowers, the carpel cleft faces the floral axis. In other single-carpellate angiosperms, the cleft of the carpel also tends to face the inflorescence or shoot axis (Leguminosae, Tucker 1984, 1988, 1992, Derstine and Tucker, 1991; Pseudowintera traversii: Winteraceae, Sampson and Kaplan, 1970; Peperomia spp.: Piperaceae, Tucker, 1980). Such evidence suggests that the principal inflorescence axis, or subtending bract influences the orientation of the carpel.

The assertion that the principal axis could direct the orientation of the carpel does not take into account the fact the flowers of Grevilleoideae are initiated on short-shoots or in pairs. It has been shown that the flowers (excluding the carpel) are dorsiventrally oriented on the short shoots. In grevilleoid taxa that have dorsiventrally oriented carpels, the carpel cleft does face the adaxial tepal of the flower, closest to the secondary short-shoot axis. In the other taxa however, the carpel can be oriented in any of five other positions (Fig. 5.4C-G). If there is an influence from other parts of the plant in these cases, the influence would have to come from some other source such as the common bract or principal inflorescence axis, not the short-shoot axis. Whether the principal inflorescence axis and/or the common bract are serving as a source of polarity directing carpel orientation, remains to be tested. Interestingly though, no observed grevilleoid taxa had a carpel cleft oriented in a lateral position facing the common bract, or facing abaxially in relation to the principal inflorescence axis.

Organizational and evolutionary implications - The foundation of systematic relationships among angiosperms relies extensively on characters in the mature flowers, primarily because the organization of structures in flowers is generally conserved among taxa. Gynoecium characters are a vital source of

taxonomic conservation at different hierarchical levels among angiosperms. The diverse orientations of the single carpels among taxa of Grevilleoideae are apparently unique among angiosperms. Such a phenomenon naturally arouses questions concerning the evolutionary implications of the flower structure. In addition, to fully appreciate the possible biological roles of variable carpel orientations in taxa of Grevilleoideae, it is essential to understand and examine the functional form of the flower including pollination syndromes, other morphological characters in the flower and or inflorescence, seed dispersal, and fruit development. There is little information concerning these life history traits in Proteaceae and therefore, postulations of associated morphological features are reviewed below.

Conservation of organization - Botanists have long been aware of the principal of conservation of organization of floral organs in flowers at various taxonomic levels. Waddington (1962) suggested that the reason for conservation of organization among flowers is due to developmental canalization: that is, certain developmental processes are less likely to change because they are tightly integrated with other developmental processes, and a change would result in a drastic reorganization and produce a dysfunctional form. Stebbins (1969, 1974) expanded on Waddington's assertion by suggesting that the conserved processes are part of the internal selection pressures of the organism and defines the integration of developmental (genetic) processes as selective inertia: "Whenever a complex, organized structure or integrated biosynthetic pathway has become an essential adaptive unit of a successful group of organisms, the essential features of this unit are conserved in all of the evolutionary descendants of the group concerned" (Stebbins, 1969: 124-125). Under the hypothesis of conservation of organization, the diversity of carpel orientations among Grevilleoideae could be the result of two different processes: firstly, multiple origins of the carpel orientations via loss of other carpels from a multicarpellate ancestor or secondly, the diverse orientations are the

result of lability of organization of the ancestral flower, followed by successively tighter integrational events in certain taxonomic groups (or successive canalization).

Multiple origins - Venkata Rao (1971) identified various carpel orientations without knowledge of the orientation of the flowers among Grevilleoideae, and subsequently proposed three hypotheses. In the first hypothesis, he asserts that the carpel orientation in Grevilleoideae flowers is dorsiventral and the flowers have variable orientations; a result of different orders of inflorescence branch reductions. He claimed that there were multiple origins of the flower pairs in Grevilleoideae via sequential secondary, tertiary, and even quarternary raceme axis reductions. Thus the orientation of the carpel in relation to the flower is dorsiventral, but the orientation of the flower in relation to the inflorescence axis varied. In a comparative ontogenetic study of the development of flower-pairs and floral orientations among Grevilleoideae, Douglas (Chapter 4) demonstrated that there are no vestigial structures that would support multiple origins of flower-pairs and that the flowers are dorsiventrally aligned on short-shoots subtended by reduced floral bracts.

Torsion - The second hypothesis Venkata Rao offered was that in some taxa the diagonally oriented carpels are the result of "torsion" during development. Developmental comparsions in the present study do not support a "torsion" process.

In the third hypothesis, Venkata Rao (1971) claimed the flower orientations were conserved and claimed that there were multiple origins of the proteaceous flower from a dichlamydeous, tetracarpellate ancestor. Thus, in taxa with diagonal carpels, there was loss of the inner perianth series, inner stamen series and three of the carpels, and the grevilleoid taxa with the carpel cleft facing a tepal were derived from the same dichlamydeous ancestor via loss of the outer perianth, the outer stamen series, and three carpels. There is no evidence for any of these morphological transformations from other

investigations (Douglas, chapter 2, 3, 4). In addition, the derivation of the flower pairs appears to be a one-time event leading to a monophyletic Grevilleoideae (Johnson and Briggs, 1975; Douglas, Chapter 6).

Successive canalization- - The single carpel among Proteaceae appears to have been a one-time phylogenetic event; it is probably a synapomorphy for the family. Nevertheless, the lack of a consistent organization within Grevilleoideae (due to carpel alignments) implies that the general integration of carpel orientations is not conserved at the inter-tribal level. There are shared developmental pathways of certain carpel orientations at tribal and some subtribal levels suggesting that certain orientations have become secondarily constrained. To support such an assertion, there should be functional adaptations associated with different carpel orientations.

Conservation of carpel orientations based on associations with other floral features - The taxonomically fixed positions of the carpel suggest that functional constraints are involved. Johnson and Briggs (1975) asserted that the diverse carpel orientations in Proteaceae are morphologically important in providing various functional symmetrical boundaries of the flowers (i.e. pollinator syndromes like pollen presentation). To fully understand the topological and developmental morphological attributes of a flower, it is essential to examine the selective and adaptive features of a plant in relation to pollination, fruit and seed dispersal. Although a comprehensive review is beyond the scope of this study, there are several morphological characters in the flowers that are associated with certain carpel orientations.

There are several floral and inflorescence features associated with the different types of carpel orientations that appear correlated with the functional expression of zygomorphy or actinomorphy. The flowers of all taxa of Grevilleoideae that lack a dorsiventral or ventral-dorsal carpel orientation are topologically asymmetrical. However,

in the context of functional symmetry in the inflorescence, the flowers do collectively express a form of symmetry. For example, Darlingia darlingiana inflorescences, like many Grevilleoids, have primary inflorescence axes that resemble a bottle-brush when the flowers open. On each flower, the perianth lobes reflex away from the straight style after the pollen has been deposited onto a specialized swollen end of the style, proximal to the stigma, known as a pollen presenter. Although the carpel is laterally oriented in Darlingia, the straight style, the total reflexion of the perianth and the fact that there are approximately 60-100 flowers on each axis that open simultaneously, makes the inflorescence and flowers functionally actinomorphic.

Correlation between ventral-dorsal carpel alignments and the tepals - Taxa with a ventral-dorsal carpel show an interesting interplay among other floral features except for Xylomelum salicinum. In all of the taxa except Xylomelum the abaxial tepal lobe is shorter than the other tepal lobes, the adaxial being the longest. The flowers of a pair curve abaxially and away from one another.

Correlation between carpel orientations and the nectaries - There appears to be a correlation between the position and type of nectary and the type of carpel orientation as well as with the inititiation of the carpel (terminal or lateral). In the taxa that have lateral carpel initiation, a large, hippocrepiform, horseshoe shaped, nectary is initiated from the floral residuum next to the cleft side of the carpel. Taxa with terminally developing carpels can have two, three, or four nectary lobes, sometimes with intercalary growth between and beneath each lobe. In proteaceous flowers, the timing of nectary initiation occurs relatively late in ontogeny, generally after the development of a stipe. In some taxa, even though the carpel initiates laterally, it will generally enlarge and take up most of the area of the remaining floral base. Nectary initiation from the floral receptacle occurs where there is an apical residuum, or where space is created as a result of secondary enlargement due to zonal growth (Douglas, Chapters 2-3; 1991).

Correlation of pre-anthesal stylar exsertion with diagonally oriented carpels - In taxa with a diagonal carpel, the middle region of the style is usually exserted from between two tepal margins prior to anthesis, the gynoecium being longer than the tepals. The style convexly bends (not arched) in genera of Macadamiinae and concavely arches in Grevilleae, Telopeae, and Banksieae. Pre-anthetic exsertion of the style occurs in some of the taxa with laterally oriented carpels as well (Buckinghamiinae, Stenocarpiinae and Lomatiinae). In Grevilleae and Telopeae, the perigon arches diagonally; the *margin* between the two tepals next to the carpel suture is the shortest. Interestingly, one side of a single tepal is a different length from the opposite side of the same tepal (Douglas, unpub.).

Phylogenetic trends in carpel ontogenesis - From developmental evidence, it can be asserted that there are conserved phylogenetic trends in carpel organogenesis among Proteaceae. Early developmental events in floral ontogeny have been demonstrated as phylogenetically informative (Hufford, 1988; Drinnan and Ladiges, 1991a,b; Tucker, Douglas and Liang, 1993; Tucker and Douglas, in press). The phylogenetic conservation of early ontogenetic processes among suprageneric taxa was hypothesized by Tucker (1984) and has subsequently been demonstrated in several angiosperm groups (Scrophulariaceae: Armstrong and Douglas, 1989; Compositae: Harris, 1991; Piperales: Tucker, Douglas and Liang, 1993; Acanthaceae: Scotland, Endress and Lawrence, 1994; Paleoherbs: Tucker and Douglas, in press; Leguminosae: Tucker, 1987; Tucker and Douglas, in press). Developmental variations that occur later in ontogeny tend to be infra-generically informative (Tucker, 1984, 1987, 1988, 1992).

There are several trends which suggest relationships among grevilleoid taxa. Firstly, if the lateral initiation of the carpel is an apomorphy, taxa of Grevilleae and Embothrieae (excluding Opisthiolepis and Lomatia) could be closely related. There are

other features that also support such a contention including the development of the short-shoot axis and floral organogenesis (Douglas, chapter 4).

The developmental events associated with the initiation of the ventral-dorsally aligned carpel of Cardwellia, Bleasdalea, and Sleumerodendrom are similar. The floral meristem during the conversion to carpel primordium becomes trapezoidal in outline, and the cleft develops ventrally. That, and the elongation of the peduncle, sequential initiation of the ventral and then dorsal nectaries, as well as the development of multipapillate hairs on the young pollen presenter (Douglas, unpub.), suggests that these genera could be closely related or at least share recent common ancestry.

Does the developmental evidence indicate the plesiomorphic carpel condition? - No, a phylogenetic analysis is necessary prior to any rash assumptions concerning the polarity of carpel orientations. In this study, I have presented a broad-based developmental comparison of carpel ontogeny among grevilleoid and other proteaceous taxa. In defining the variation, I have suggested a few testable trends in carpel ontogeny and morphogenesis. The aim of this paper is to document carpel ontogenesis and carpel orientations among grevilleoid flowers, prerequisites to establishing phylogenetic characters.

LITERATURE CITED IN CHAPTER 5

Armstrong, J. E. and A. W. Douglas. 1989. The ontogenetic basis for corolla aestivation in Scrophulariaceae. Bulletin of the Torrey Botanical Club 116: 378-389.

Bailey, I. W. and B. G. L. Swamy. 1951. The conduplicate carpel of dicotyledons and its initial trends of specialization. American Journal of Botany 38: 373-378.

Baum, H. 1952. Der Bau des Karpellsstiels von Grevillea thelemanniana und seine für die Beurteilung der Epeltaten Karpelle. Phytomorphology 2: 191-197.

Briggs, B. G. and L. A. S. Johnson. 1979. Evolution in the Myrtaceae - Evidence from inflorescence structure. Proceedings of the Linnean Society of New South Wales 102: 157-272.

Brooks, R. M. 1940. Comparative histogenesis of vegetative and floral apices in Amygdalus communis, with special reference to the carpel. Hilgardia 13: 249-299.

Crisp, M. D. and P. H. Weston. 1987. Waratahs--How many species? Australian National Botanic Gardens (Occ. Publ.), 9: 3-15.

Derstine K. S. and S. C. Tucker. 1991. Organ initiation and development of inflorescences and flowers of Acacia baileyana. American Journal of Botany 78: 816-832.

Douglas, A. W. 1989. The ontogenetic basis of diverse floral morphologies in Pedicularis L. (Scrophulariaceae). M.S. Thesis. Illinois State University, Normal, Illinois.

Drinnan, A. N. and P. Y. Ladiges. 1991a. Floral development and systematic position of Eucalypus curtisii (Myrtaceae). Australian Systematic Botany 4: 539-551.

Drinnan, A. N. and P. Y. Ladiges. 1991b. Floral development in the 'Symphyomyrtus Group' of Eucalypts (Eucalyptus: Myrtaceae). Australian Systematic Botany 4: 553-562.

Endress, P.K. 1972. Zur vergleichenden Entwicklungsmorphologie, Embryologie un Systematic bei Laurales. Botanische Jahrbüscher der Systematik 92: 331-428.

Endress, P.K. 1987. The Chloranthaceae: Reproductive structures and phylogenetic position. Botanische Jahrbücher der Systematik 109: 153-226.

Engler A. in Engler, A. and K. Prantl. 1894. Die Naturlichen Pflanzenfamilien. Leipzig. Excerpt on Proteaceae by A. Engler (1893), Vol IV.

Friis, E.M. and P.K. Endress. 1990. Origin and evolution of angiosperm flowers. Advances in Botanical Research 17: 99-162.

Fuss, A.M. and M. Sedgley. 1991. Floral initiation and development in relation to the time of flowering in Banksia coccinea R.Br. and B. menziesii R.Br. (Proteaceae). Australian Journal of Botany 38: 487-500.

Goethe, J. W. 1790. Versuch der Metamorphose der Pflanzen zu erklären. Gotha.

Gregoire, V. 1938. La morphogenese et l'autonomie de l'appareil florale. I. Le carpelle.

Haber, J. M. 1959. Comparative anatomy and morphology of flowers and inflorescences of the Proteaceae I. Some Australian taxa. Phytomorphology 9: 325-358.

Haber, J. M. 1961. The comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae II. Some American taxa. Phytomorphology 11: 1-16.

Haber, J. M. 1966 Comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae III. Some African taxa. Phyomorphology 16: 490-527.

Harris, E. M. 1991. Comparative inflorescence and floral development in the Asteraceae. Ph. D. Dissertation. Louisiana State University, Baton Rouge, LA.

Heel, W. A. van. 1981. A S.E.M.-investigation on the development of free carpels. Blumea 27: 499-522.

Heel, W. A. van. 1983. The ascidiform early development of free carpels, a S.E.M.-investigation. Blumea 28: 231-270.

Heel, W. A. van. 1984. Variation in the development of ascidiform carpels, an S.E.M.-investigation. Blumea 29: 443-452.

Hufford, L. D. 1988. Roles of early ontogenetic modifications in the evolution of floral form of Eucnide (Loasaceae). Botanische Jahrbüscher der Systematik 109: 289-333.

Johnson, L. A. S. and B. G. Briggs. 1963. Evolution in the Proteaceae. Australian Journal of Botany 11: 21-61.

Johnson, L. A. S. and B. G. Briggs. 1975. On the Proteaceae--the evolution and classification of a southern family. Botanical Journal of the Linnaean Society 83-182.

Leins, P. and C. Erbar. 1982. Des monokarpellate Gynoeceum von Monodora crispata Annonaceae. Beitrage zur Biologie der Pflanzen 57: 1-13.

Newman, I. V. 1934. Studies in the Australian Acacia. III. Supplementary observations on the habit, carpel, spore production and chromosomes of Acacia baileyana (F.v.M). Proceedings of the Linnean Society of New South Wales 59: 237-251.

Newman, I. V. 1936a. Ontogeny of the angiospermic carpel. Nature 137: 70-71.

Newman, I. V. 1936b. Studies in the Australia Acacias. VI. The meristematic activity of the floral apex of Acacia longifolia and A. suaveolens as a histogenetic study of

the ontogeny of the carpel. Proceedings of the Linnean Society of New South Wales 61: 56-88.

Payer, J.B. 1857. Traité d'Organogénie comparée de la fleur. Paris: Librairie de Victor Masson.

Periasmy and B. G. L. Swamy. 1956. The conduplicate carpel of Cananga odorata. Journal of the Arnold Arboretum 37: 366-372.

Posluzny, U. 1981. Unicarpellate floral development in Potamogeton zosteriformis. Canadian Journal of Botany 59: 495-504.

Sampson, F. B. and D. R. Kaplan. 1970. Origin and development of the terminal carpel in Pseudowintera traversii. American Journal of Botany 57: 1185-1196.

Scotland, R. W., P. K. Endress and T. J. Lawrence. 1994. Corolla ontogeny and aestivation in the Acanthaceae. Botanical Journal of the Linnean Society 114: 49-65.

Stebbins, G. L. 1969. The basis of progressive evolution. University of North Carolina Press, Chapel Hill.

Stebbins, G. L. 1974. Flowering Plants. Evolution above the species level. Cambridge, Harvard University Press.

Thompson, J. M. 1936a. On the gynoecial apex and the terminal legume of Acacia longifolia and A. suaveolens. Publications of the Hartley Botanical Laboratory (Liverpool University) 16: 3-36.

Thompson, J. M. 1936b. Ontogeny of the angiospermic carpel. Nature 137:458-459.

Tucker, S. C. 1959. Ontogeny of the inflorescence and the flower in Drimys winteri var chilensis. University of California Publications in Botany 30: 257-336.

Tucker, S. C. 1980. Inflorescence and flower development in the Piperaceae. I. Peperomia. American Journal of Botany, 67: 686-702.

Tucker, S. C. 1984. Origin of symmetry in flowers. Pages 351-395 *in* R. A. White, and R.C. Dickison, eds. Contemporary problems in plant anatomy. Academic Press, New York.

Tucker, S. C. 1987. Floral initiation and development in legumes. Pages 183-239 *in* C.H. Stirton, ed. Advances in legume systematics, Part 3. Royal Botanic Gardens, Kew.

Tucker, S. C. 1988. Loss versus suppression of floral organs. Pages 69-82 *in* P. Leins, S. C. Tucker, and P. K. Endress, eds. Aspects of floral development. Gebrüder Borntraeger, Stuttgart.

Tucker, S. C. 1992. The role of floral developmental in studies of legume evolution. Canadian Journal of Botany 70: 692-700.

Tucker, S. C., Douglas, A. W. and Liang, H. -X. 1993. Utility of ontogenetic and conventional characters in determining phylogenetic relationships of Saururaceae and Piperaceae (Piperales). Systematic Botany, 18: 614-641.

Tucker, S. C. and A. W. Douglas. In Press. Floral structure, development, and relationships of paleohers: Saruma, Cabomba, Lactoris and selected Piperales. *In* L. Hickey and D. W. Taylor, eds. Flowering plant origin, phylogeny, and evolution.

Tucker, S. C. and Gifford, Jr. E. M. 1966a. Organogenesis in the carpellate flower of Drimys lanceolata. American Journal of Botany 53: 433-442.

Tucker, S. C. and E. M. Gifford, Jr. 1966b. Carpel development in Drimys lanceolata. American Journal of Botany 53: 671-678.

Venkata Rao, C. 1957. Cytotaxonomy of the Proteaceae. Proceedings of the Linnaean Society. N.S.W. 82: 257-271.

Venkata Rao, C. 1961. Studies in the Proteaceae II. Tribes Placospermeae and Conospermeae. Proceedings of the National Institute of Sciences, India. 27B: 126-151.

Venkata Rao, C. 1966. Studies in the Proteaceae VII. Floral anatomy of Hakea Schrad. Journal of the Indian Botanical Society 45: 129-140.

Venkata Rao, C. 1971. Proteaceae. Botanical Monograph #6. Council of Scientific and Industrial Research. New Delhi.

Waddington, C. H. 1962. New patterns in genetics and development. Columbia University Press, New York.

Weberling, F. 1985. Zur Infloreszenzmorphologie der Lauraceae. Botanische Jahrbücher der Systematik 107: 395-414.

Weberling, F. 1989. Morphology of Flowers and Inflorescences. Cambridge University Press, Great Britain.

CHAPTER 6

PHYLOGENETIC ANALYSIS OF PROTEACEAE USING FLORAL ONTOGENETIC EVIDENCE

INTRODUCTION

Proteaceae is a moderate sized, natural family comprising approximately 75 genera (sensu Johnson and Briggs, 1975; 72 based on generic circumscriptions of Weston, 1983, 1991; Crisp and Weston, 1987; and Rourke, 1984b) and approximately 1200 species. Most of the taxa are limited to the southern hemisphere with centers of diversity in Australia, Africa and South America. Greatest species diversity and richness are present in the sclerophyll communities of Australia and Africa (particularly western Australia), and the greatest generic diversity is found in the tropical rainforests of Australia, and neighbouring Malesian provinces (Johnson and Briggs, 1975).

There is a high degree of morphological diversity in the flowers, inflorescences and fruits of Proteaceae. Using floral and fruit characters, Brown (1810) was the first to establish a two-subfamilial classification (seven tribes; 38 genera) of Proteaceae based on the indehiscent or dehiscent nature of the fruits: Nucamentaceae and Folliculares. Engler (1894) modified the names to Persoonioideae and Grevilleoideae, and added the character of two flowers per common bract axil for Grevilleoideae. Until 1963, the classification of Brown (1810) remained the accepted one. Johnson and Briggs (1963) reexamined the relationships of Proteaceae and included assorted new taxa that had been discovered in the 150-year interim since Brown's (1810) work. In their comparative study, they maintained the two-subfamilial ranking of the family and added four tribes to Grevilleoideae. Johnson and Briggs (1975) established a new phylogeny with five subfamilies, using karyological, floral, inflorescence, and fruit characters: Persoonioideae (7 genera; collapsed to 4 by Weston, 1983), Sphalmioideae (monotypic), Carnarvonioideae (two species), Proteoideae (26 genera) and Grevilleoideae (40 genera; raised to 41 by Crisp Weston, 1987). Johnson and Briggs (1975) grouped the taxa based on a modified pairwise comparison analysis. Character derivations (polarizations) were based on the general conditions from a deduced hypothetical "proto-proteaceous taxon." In their

currently accepted classification, the genera are classified into distinct subtribes, tribes and subfamilies. There is however little resolution of phylogenetic relationships among the taxonomic groups at all levels, and they assert that the monophyly of supra-generic rankings could change following more detailed morphological analysis of flowers and vegetative features of the plants.

Many of the problems surrounding classifications of the family, as well as the relationship of Proteaceae to other angiosperm groups, involve the peculiar and unique flowers and inflorescences. The flowers of Proteaceae have a conserved and bizarre floral topology of four free valvate tepals, four basifixed anthers, one of which is opposite each tepal member (superpositioned) and is usually adnate to the tepal, and a single carpel. Johnson and Briggs (1975) identified several morphologically diverse features of proteaceous flowers that confound the determination of homology among taxa: the perianth, the homology of the nectaries, the flower pairs in Grevilleoideae, the basis of symmetry and the orientation of the flower and/or carpels. In an ongoing project investigating the developmental basis of floral diversity in Proteaceae, floral ontogenetic studies have revealed new evidence of the topological similarities among floral organs as well as hypothetical transformational series of flowers and inflorescences in the family. Using an ontogenetic comparative approach (Chapters 2-5), the fundamental components involved with floral diversity and the homology of organization among the flowers have been ascertained. Evidence of homology of organization of the perianth, nectaries, flower-pairs, and carpel orientations among taxa, is incorporated in a phylogenetic analysis of the family. Ontogenetic investigations of floral topologies have provided a new perspective concerning the floral diversity in the subfamily Grevilleoideae and is included within this context.

The primary aim of this paper is to establish a working phylogeny of Proteaceae using morphological characters based on the principles of parsimony. In addition to

establishing a phylogeny of the family, there are two other goals of this paper: firstly, to provide evidence for or against the monophyletic status of existing subtribes, tribes and subfamilies (sensu Johnson and Briggs, 1975); and secondly, to examine the evolutionary divergence and phylogenetic origins of some of the problem characters including; 1) the nectary, 2) the carpel orientations and positions and 3) the flower-pairs and inflorescences. The phylogeny will serve as a working hypothesis that will most likely change as additional data is compiled, based on future floral ontogenetic comparisons.

METHODS AND MATERIALS

Taxa - Taxa included in the analyses and provenance are listed in Table 6.1. A total of 53 proteaceous taxa (49 genera) are included in the analysis for which relatively complete data could be obtained. Material was fixed in FAA and stored in 60% ethanol. Ontogenetic analyses and comparisons were done with scanning electron microscopy following microdissection of developing flowers as described in Douglas (Chapters 2-5).

Outgroups - Proteaceae is a distinct and natural family (Johnson and Briggs, 1975) although its relationship with other angiosperm families is unclear. In general, there are two schools of thought concerning proteaceous affinities to other angiosperms, both of which are based on interpretations of the unique floral morphology. The first school identifies Proteaceae as a distnct and highly derived family, the flowers of which are the product of reduction from a dichlamydeous ancestor, primarily by reduction of a petal series to nectaries, and reduction to a pseudomonomerous carpel. Dichlamydeous angiosperm groups from which Proteaceae has been hypothetically derived include Thymeleaceae by Bentham and Hooker, 1883; Rosales/Sapindales by Hallier, 1912; Sapindales by Bessey, 1915; Aristolochiales/Myrtales/Santalales/Thymeleales by Pulle, 1950; Santalales by Lawrence, 1954; Thymeleaceae by Hutchinson (1959) and Eames

Table 6.1. Taxa included in the analysis and provenance.

Taxa	Native to:	Source
Platanus occidentalis L.	Eastern U.S.	Baton Rouge, LA
Sabia racemosa Chen.	Borneo, Malaysia	Harvard Herbarium
Placospermum coriaceum C.T. White & Francis	N.E. Queensland	N.E. Queensland
Garnieria spathulifolia Brongn.& Gris.	New Caledonia	New Caledonia (Weston, coll.)
Persoonia myrtilloides Sieb. ex Schult. & Schult. F.	S.E. Australia	University of California at Santa Cruz (UCSC) arboretum
Persoonia falcata R. Br.	E. Australia	E. Australia, Queensland
Bellendena montana R. Br.	Tasmania	Tasmania, three collectors (Alf Salkin, Peter Jobson, and Jennifer Chappill)
Adenanthos obovatus Labill.	S. W. Australia	UCSC arboretum; Stirling Range, W. Australia.
Adenanthos oreophilus Nelson	S. W. Australia	Royal Botanic Gardens (R.B.G.) Sydney
Cennarhenes nitida Labill.	Tasmania	Tasmania (F. Podyer, coll.; CSIRO collection, Atherton, Queensland)
Beaupreopsis paniculata (Brongn. & Gris.) Virot	New Caledonia	New Caledonia, (Weston, coll.)
Agastachys odorata R. Br.	Tasmania	Tasmania (J. Chappill, coll.)
Symphionema montana R. Br.	S. E. Australia	R.B.G. Sydney - Mt. Annan Gardens
Stirlingia latifolia Steud.	S. W. Australia	Stirling Range, S. W. Australia
Petrophile serruriae R. Br.	S. W. Australia	U.C.S.C. arboretum
Isopogon formosus R. Br.	W. Australia	U.C.S.C. arboretum
Conospermum caeruleum R. Br.	W. Australia	U.C.S.C. arboretum; Western Australia.

con'd

Taxa	Native to:	Source
Synaphea polymorpha R. Br.	W. Australia	R.B.G. Sydney- Mt. Annan Gardens
Faurea saligna Harv.	South Africa	Ristenburg district (Hugh Glen; R.L.Chapman, colls.)
Protea neriifolia R. Br.	South Africa	U.C.S.C. arboretum
Vexatorella obtusata Rourke	South Africa	Kirstenbosch gardens, South Africa (T. McLellan, coll.)
Leucospermum reflexum Buek., ex Meissn.	South Africa	U.C.S.C. arboretum
Mimetes fimbriaefolia Knight.	South Africa	Kirstenbosch gardens. South Africa (T. McLellan, coll.)
Serruria pedunculata R. Br.	South Africa	U.C.S.C. arboretum
Carnarvonia aralifolia F. Muell.	N.E. Queensland	CSIRO arboretum, Queensland; N.E. Queensland (B. Hyland, coll.)
Sphalmium racemosum (C.T. White) B.Briggs, B. Hyland, L. Johnson	N.E. Queensland	N.E. Queensland (T. Irvine, coll.); R.B.G. Kew, London, England (B. Hyland, coll.)
Orites revoluta R. Br.	Tasmania	Tasmania, (J. Chappill, coll.)
Neorites kevediana L.S. Smith	N.E. Queensland	N.E. Queensland, B.Gray (coll); R.B.G. Kew, London, England, (B. Hyland, coll.)
Darlingia darlingiana (F. Muell.) L. Johnson	N.E. Queensland	N.E. Queensland; T. Irvine's private arboretum
Cardwellia sublimis F. Muell.	E. Queensland	N.E. Queensland
Grevillea baileyana McGillivray	N.E. Queensland	N.E. Queensland
Grevillea vestita (Endl.) Meissner	S.W. Australia	U.C.S.C. arboretum
Hakea myrtoides Meissner	S.W. Australia	U.C.S.C. arboretum
Banksia praemorsa Andrews	S.W. Australia	U.C.S.C. arboretum
Dryandra polymorpha George	S.W. Australia	U.C.S.C. arboretum

con'd

Taxa	Native to:	Source
Austromuellera trinerva C.T. White	N.E. Queensland	N.E. Queensland
Musgravea stenostachys L.S. Smith	N.E. Queensland	N.E. Queensland
Opisthiolepis heterophylla L.S. Smith	N.E. Queensland	N.E. Queensland, T. Irvine's private arboretum
Buckinghamia celsissima F. Muell.	E. Queensland	James Cook University Arboretum, (Peter xxxx coll.)
Telopea speciosissima (Sm.) R. Br.	E. Australia	U.C.S.C. Arboretum
Embothrium coccineum Forst.	Chile, South America	U.C.S.C. Arboretum; R.B.G. Sydney (P. Weston, coll.)
Alloxylon flammeum P.H. Weston & M.D. Crisp	N.E. Queensland	N.E. Queensland
Stenocarpus salignus R. Br.	Eastern and N.E. Australia	Strybing arboretum, San Francisco, California.
Hollandaea sayerana (F. Muell.) L.S. Smith	N.E. Queensland	N.E. Queensland (B. Hyland, coll.)
Triunia erythrocarpa D. Foreman	N.E. Queensland	N.E. Queensland
Bleasdalea bleasdalei (Syn. Turillia) (F. Muell.) A.C. Smith	Eastern Queensland	Eastern Queensland, Mt. Spec.
Gevuina avellana Molina	Chile, South America	U.C.S.C. Arboretum
Macadamia integrifolia Maiden & Betche	E. Australia	R.B.G. Sydney; U.C.S.C. arboretum; University of Melbourne; Waimea Arboretum, Hawaii (coll.)
Brabejum stellatifolium L.	South Africa	Kirstenbosch Gardens, S. Africa (T. McLellan, coll.. and D. Keats, coll.)
Roupala montana Aubl.	South and Central America	French Guiana, South America (E.M. Harris & M.F. Quigley, coll.); Costa Rica (M. Wiemann, coll.)

con'd

Taxa	Native to:	Source
Lambertia formosa Sm.	S.W. Australia	U.C.S.C. Arboretum
Lambertia inermis R. Br.	S.W. Australia	U.C.S.C. Arboretum
Lomatia fraxinifolia R. Br.	E. Australia	Strybing Arboretum; Missouri Botanical Gardens, Herbarium (coll.)
Helicia lamingtonia (F.M. Bail.) C.T. White	N. E. Queensland	N.E. Queensland (B. Gray, coll.)
Athertonia diversifolia (C.T. White) L.S. Johnson & B. Briggs	N.E. Queensland	N.E. Queensland.

(1961); Leguminosae and Thymeleanae by Dahlgren, 1975; Elaeagnaceae by Cronquist, 1968, 1988; Santalaceae/Loranthaceae and Balanophoraceae by Venkata Rao, 1971; and Leguminosae by Thorne, 1968. The second school of thought concerning the derivation of Proteaceae places emphasis on the apocarpous nature of the gynoecium in Proteaceae, a primitive condition of angiosperm flowers. It is suggested that the flowers of the family represent those of an early derived angiosperm lineage, that are analogous to "nature's *experimental stages* in the evolution of more derived dichlamydeous taxa" (italics in quotes in Venkata Rao, 1971). Taxonomists supporting this concept of Proteaceae as relatively primitive have derived Proteaceae from Ranunculales (Proberberidaceae of Engler, 1893) and allied the group in some cases with Santalales (Rendle, 1959) or as having uncertain affinities according to Wettstein (1935). Johnson and Briggs (1975) support the contention that Proteaceae represents a primitive angiosperm family, based on comparative morphological analyses. They assert that Proteaceae represents a pre-Rosidae lineage. Thus they consider that the family is primitively apocarpous and is derived from an ancestor that had little or no differentiation of perianth parts, so is primitively simple.

A consensus among morphologists who have worked with select taxa of Proteaceae in comparative angiosperm studies is that Proteaceae stands alone, having had a long and isolated history. As a result, Proteaceae cannot be allied with any one angiosperm group based on morphological characters alone. Comparative morphological studies include: the adaptive peaks of correlated floral features among angiosperms by Stebbins (1951); leaf architecture comparisons by Hickey and Wolfe (1975); as an early Glossopteridae offshoot based on leaf characters by Melville (1975); and morphological and anatomical anther comparisons in Rosidae by Endress and Stumpf (1991).

The family Sabiaceae has been suggested as the sister group to Proteaceae by Chase et al. (1993). In the same study, Proteaceae/Sabiaceae represent a basal clade of the Hamamelids. Chase et al. (1993) compared DNA sequences of ribulose bisphosphate

carboxylase oxygenase/reductase (RUBISCO, chloroplast encoded large subunit). Only one taxon from both Sabiaceae and Proteaceae were used in the Chase et al (1993) analysis. D. Soltis (pers. comm.) has sequenced several additional proteaceous taxa and Sabiaceae (sensu Cronquist, 1988), and contends that the position of Proteaceae appears relatively stable as a basal hamamelid with the Malaysian Sabia (Sabiaceae sensu stricto; Heywood, 1978) as the sister taxon. The other two tropical american genera of Sabiaceae, sometimes classified in the family Meliosmaceae (Heywood, 1978), are Meliosma and Ophiocaryon. These taxa appear to be more closely related to sapindalean taxa among Rosidae. The sister clade to Proteaceae/Sabiaceae is Nelumbonaceae/Platanaceae in Chase et al. (1993). This four-familial clade is a node away from the more basal (?) Ranunculales, and two nodes from Magnoliidae and the base of the angiosperms. Therefore, Proteaceae could represent a very early angiosperm lineage, as suggested by Wettstein (1935), Rendle (1959) and Johnson and Briggs (1975).

Because of the numerous hypotheses about ancestors to Proteaceae, and the fact that multiple outgroups tend to result in ambiguous polarizations and optimizations of the characters in the ingroup (Nixon and Carpenter, 1993; Tucker and Douglas, in press), the taxonomic relationship hypotheses from DNA sequence parsimony analyses are used (Chase et al, 1993; Soltis pers. comm) to select outgroups. Outgroups in the analysis include Platanaceae (Platanus occidentalis) and Sabiaceae (Sabia gracilis). Platanaceae is included because the family has been hypothesized as the basal member of the Hamamelidae based on molecular evidence (Chase et al, 1993) and in a morphological phylogenetic analysis by Schwarzwalder and Dilcher (1991). In all analyses, the outgroup and ingroup taxa were analyzed simultaneously (simultaneous outgroup analysis sensu Farris, 1970; Meacham, 1984; Nixon and Carpenter, 1993). The unrooted network(s) was then rooted with the outgroup taxa, and subsequent character polarity was

examined after selected optimizations (delayed or accelerated transformations). This method ensured most-parsimonious trees as defined by Farris (1970, 1982).

Cladistic analyses - Phylogenetic analyses were carried out with both Hennig 1.5 (Farris, 1988) and PAUP 3.1 (Swofford, 1991). Tree manipulations and character distributions were examined with CLADOS (vers. 1.2; Nixon, 1992) and MacClade (Maddison and Maddison, 1992). All multistate characters were unordered (non-additive). Approximation parsimony methods are used here because the data matrix is quite large (options 'mh* and bb*' in Hennig86, and 100 random starting point heuristic searches in PAUP 3.1).

Data shortcomings - Missing data are present in different forms and for different reasons in the data matrix (Appendix 1). In some cases, character states for certain characters were not available for the analysis (e.g. leaf vascular characters of centric leaves in sclerophyllous taxa; pollen characters, 148-152). In these cases, the missing characters were coded as unknown. In other cases, assessment of homology of characters among taxa was difficult (e.g. the variation of features intrinsically connected to flower pairs of Grevilleoideae, characters 94, 95, 98 99 101). Thus, in taxa where there is difficulty in determining the homologous state, the taxa are coded as 'uncertain' for that character (Bremer, 1991). A problem in phylogenetic algorithms is that the inclusion of characters or taxa with a high proportion of 'uncertain' or 'unknown' characters in the matrix can result in misleading topologies (Nixon and Davis, 1991; Platnick et al. 1991). In order to examine the effects of the missing data in parsimony analyses, the data were reanalyzed after characters that had a high proportion of missing values were excluded as separate groups (second analysis) and entirely (third analysis).

Cladogram stability - There are various methods of examining the stability of clades within a parsimonious tree. One technique used here is decay analysis as defined

by Bremer (1988). In decay analysis, a heuristic search using PAUP was executed, and the total trees five steps greater than the most parsimonious tree(s) were retained (MULPARS option in affect). A strict consensus of total trees 5 steps longer was calculated and clades or nodes supported with 5+ characters were recorded. The 5+-trees were then filtered and only trees that were four or less steps were retained. Another strict consensus tree was generated and the clades supported by four-step additions were recorded. The process of filtering, consensus, and recording continued one step at a time until the most-parsimonious tree(s) were left.

Characters - A total of 154 characters are included in this analysis (Appendices 1, 2). Characters are established based on ontogenetic comparisons of morphological features, comparisons of the mature and preanthesis inflorescences, and flowers and leaf architecture characteristics. Additional data were obtained from studies of pollen characters by Erdtman (1952) and Feuer (1986, 1989, 1990), wood anatomy by Chattaway, (1948), Mennega (1966), Lanyon (1979); anatomical and embryological characters by Venkata Rao (1957 et seq.); and morphological characters by Johnson and Briggs (1963, 1975). Data for Platanaceae were obtained from Schönland (1883), Bretzler (1924), Boothroyd (1930) and Schwarzwalder and Dilcher (1991).

After establishing a monophyletic group, character assessment is perhaps the most important aspect of phylogenetic systematics and cladistic methodologies (Stevens, 1991). Ontogenetic analyses are necessary to assessing the phylogenetic significance of morphological characters in three ways; firstly ontogenetic comparative studies provide independently assessed organizational qualities of flowers. They also provide evidence of structural homology or topological similarity among characters without preconceived notions of affinities (Kaplan, 1984; Patterson, 1982, 1987). Secondly, ontogenetic comparisons provide a means of empirically examining transformation hypotheses involved in the disparate forms. Thirdly, comparative ontogenetic analyses often reveal

convergences among structures and processes involved with morphological diversification.

Within an ontogenetic framework, it is possible to determine the fundamental elements of organization and thus independently assess structural homology (topological similarity, Patterson, 1982) among taxa or organs of taxa (Wardlaw, 1965; Kaplan, 1984; Tucker, Douglas and Liang, 1993). I reviewed characters in Johnson and Briggs (1975). Using ontogenetic comparisons of available taxa and evidence of topological similarity, I recoded and in some cases divided characters. For example, the character "curved or arched style" is divided into two characters: dorsal curvature of the style (the style arching away from the cleft side of the carpel) and ventral curvature of the style (the style arching towards the cleft side of the carpel), based on the fact that the process of curvature differs in its site and action in the two.

Several original ontogenetic characters were included in the analysis. There are two types of developmental characters as defined by Tucker, Douglas and Liang (1993). **Ephemeral characters** are characters whose states are present at some stage in the ontogeny of the flower and are later disguised by other differentiation processes. Examples include the position of the carpel at initiation, character 59, or the presence of multipapillate hairs on the upper portion of the style that are not present at anthesis, character 65. The second type of character derived from ontogenetic comparisons is the **persistent characters** that have states identifiable in the mature flower, although the differentiation processes of the states can be recognized through developmental time. For example, the ovule position/displacement character (82) includes the processes that give rise to transversely arranged ovules within the loculus, and the stigma position character (78) includes the differential enlargement processes of the carpel that result in the stigma being positioned on the abaxial side of the carpel. Ontogenetically derived characters used in this analysis are indicated with *italics* in Appendix 2. Relatively few ontogenetic

characters are employed within this phylogenetic analysis, as complete ontogenetic series were unavailable for some taxa. Future ontogenetic investigations should provide more characters and clarification of characters.

Leaves were cleared using a modified method of Foster (1952). Leaves were cleared in a 5% aqueous sodium hydroxide solution at approximately 45 degrees celsius for one to 20 days depending on the leaf. It should be noted that most proteaceous leaves turn pitch black when put in sodium hydroxide. Leaves were then carefully transferred to a distilled, deionized, deoxygenated water bath. Following the wash, leaves were put on a large glass sheet with water and used as a negative on a Besseler 4x5 photographic enlarger. Depending on the size of the leaf, either a 50 mm or 135 mm enlarging lens was used to transfer the venation image to Kodak Polycontrast IIrc developing paper. This method captured the primary architecture in the large leaves. Following exposure, the cleared leaves were transferred to a chloral hydrate solution until cleared, washed in water (5 changes), dehydrated in 50% ethanol, stained in safranin for 30 seconds to 5 minutes depending on the leaf, and dehydrated through an ethanol series to absolute ethanol. Following dehydration, the leaves were transferred to an 1:1 solution of ethanol and toluene, and then to a solution of pure toluene. Leaves too large for a 2"x4" slide were cut into small pieces and were then mounted on glass slides with permount and a coverslip.

RESULTS

From the initial analysis which included 154 characters and 55 taxa, 22 most-parsimonious trees were obtained (1180 steps; c.i.=.247; r.c.i.=.148; r.i.=.59; strict consensus shown in Fig. 6.1). In the strict consensus tree (Fig. 6.1), there is a lack of resolution among the basal clades of Grevilleoideae (note the five-branched polytomy or bush).

Figure 6.1. Strict consensus of 22 most parsimonious trees using all 154 characters. The trees were each 1180 steps (c.i.=0.247; r.c.i.=0.148; r.i.=0.59). Taxa of Persoonioideae are in **bold-face**; taxa of Proteoideae are in regular type, as are the unigeneric subfamilies, Sphalmioideae and Carnarvonioideae; taxa of Grevilleoideae are underlined and have wider branches. There is a five-branched polytomy or bush among Grevilleoideae above Neorites.

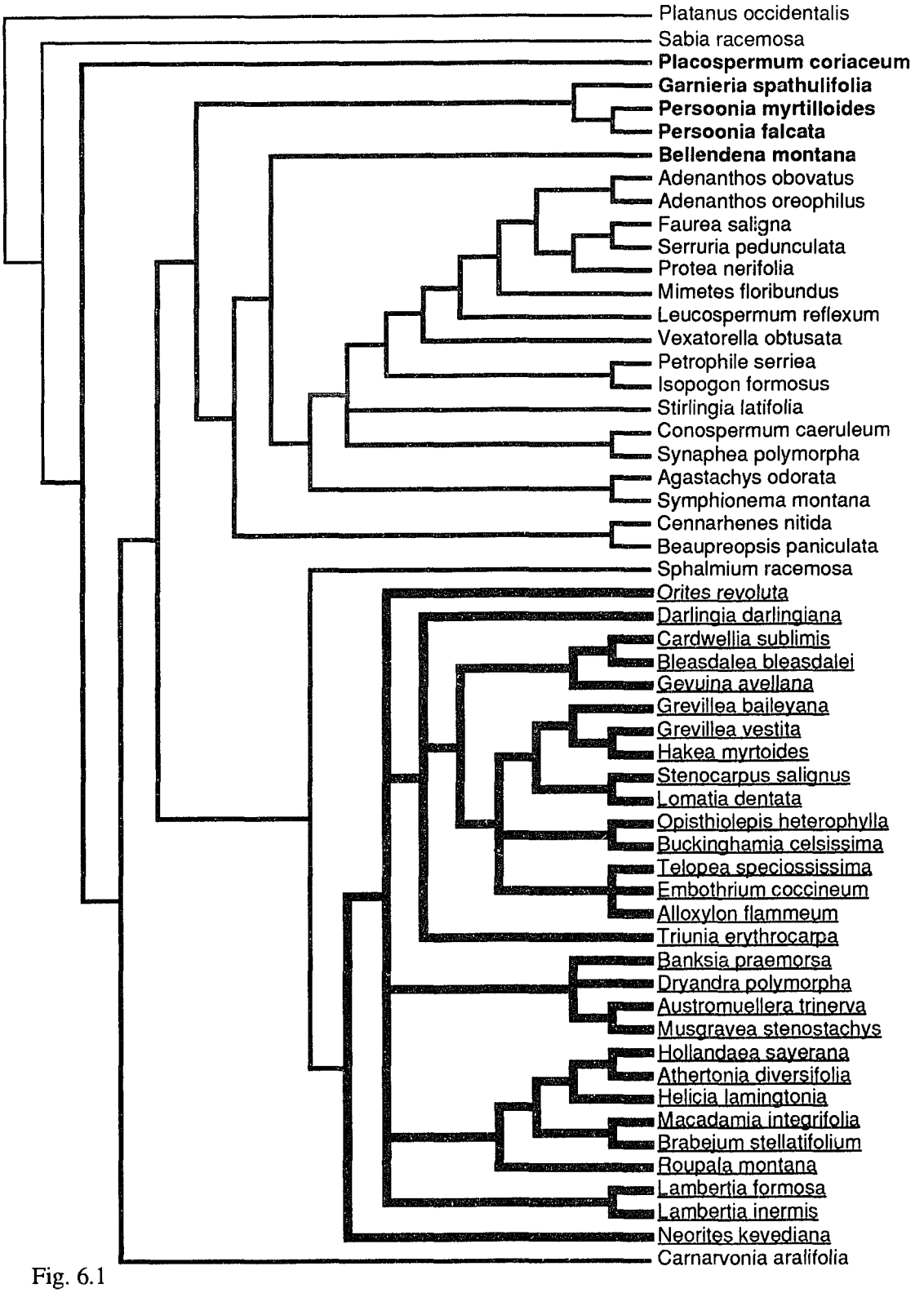

Platanus occidentalis
Sabia racemosa
Placospermum coriaceum
Garnieria spathulifolia
Persoonia myrtilloides
Persoonia falcata
Bellendena montana
Adenanthos obovatus
Adenanthos oreophilus
Faurea saligna
Serruria pedunculata
Protea nerifolia
Mimetes floribundus
Leucospermum reflexum
Vexatorella obtusata
Petrophile serriea
Isopogon formosus
Stirlingia latifolia
Conospermum caeruleum
Synaphea polymorpha
Agastachys odorata
Symphionema montana
Cennarhenes nitida
Beaupreopsis paniculata
Sphalmium racemosa
Orites revoluta
Darlingia darlingiana
Cardwellia sublimis
Bleasdalea bleasdalei
Gevuina avellana
Grevillea baileyana
Grevillea vestita
Hakea myrtoides
Stenocarpus salignus
Lomatia dentata
Opisthiolepis heterophylla
Buckinghamia celsissima
Telopea speciossissima
Embothrium coccineum
Alloxylon flammeum
Triunia erythrocarpa
Banksia praemorsa
Dryandra polymorpha
Austromuellera trinerva
Musgravea stenostachys
Hollandaea sayerana
Athertonia diversifolia
Helicia lamingtonia
Macadamia integrifolia
Brabejum stellatifolium
Roupala montana
Lambertia formosa
Lambertia inermis
Neorites kevediana
Carnarvonia aralifolia

Fig. 6.1

The presence of unknown and uncertain characters in the analysis appeared to affect the resolution among taxa in the subfamily Grevilleoideae. Because the missing values in the pollen-character subset (unknown) are the highest (~ 33% among Grevilleoideae), the pollen characters (148-152) were excluded from the second analysis. Six most parsimonious trees were obtained (1146 steps; c.i.=.25; r.c.i.=.15; ri=.60). Resolution was much improved, with only three polytomies (Fig. 6.2) among those found in the first analysis that used total data (Fig. 6.1). In the 50% majority consensus tree of the second analysis (unsupported nodes with arrows; Fig. 6.2) there are three clades that are differentially resolved among the six trees; the position of Stirlingia either above or below Conospermum/Synaphea; Banksiinae as a monophyletic group in four of the six trees and representing a grade in the other two; and Lambertiinae that was resolved as a sister clade to Banksieae in four trees and as the sister clade to Roupala and other Macadamieae in the other two. The majority rule tree had the same topology as one of the six trees except for Stirlingia.

A third analysis of the data excluded the 'uncertain' characters (flower-pair characters, 94, 95, 98, 99, 101) as well as the 'unknown' pollen characters resulted in two most parsimonious trees (one tree shown in Fig. 6.2: 1127 steps, c.i.=.25, r.c.i.=.15; r.i.=.60). The difference between the two trees is in the position of Stirlingia either as a node above or below the Conospermum/Synapheae clade (** in Fig. 6.2). Lambertiinae is resolved as the sister group to Banksieae, and Banksiinae (sensu Johnson and Briggs, 1975) is paraphyletic. Both tree topologies were present in the first and second analysis.

The missing values among taxa could be providing topological resolution based on the numerous parsimonious and ambiguous arrangements that are not empirically supported. Several studies have defined and identified similar problems in data analysis (Platnick et al. 1991; Nixon and Wheeler, 1992; Bruneau and Doyle, 1993). The

Figure 6.2. One of six most parsimonious trees obtained from an analysis that excluded the 'unknown' or missing pollen characters (1146 steps; c.i.=0.25; r.c.i.=0.15; r.i.=0.59). The same tree was one of two trees obtained from the third analysis that excluded the pollen characters and the 'uncertain' characters involved with the flower pairs. In both the second and third analysis, the multiple tree topologies matched tree topologies from the first analysis. Decay indices are indicated above branches. In addition, the arrows and double hash marks indicate clades that were not supported in all of six trees. In the second analysis, the position of Stirlingia varied between a basal or a derived node (below or above) the Conospermum/Synaphea clade. Taxa of Persoonioideae are in **bold-face**; taxa of Proteoideae are in regular type as are the unigeneric subfamilies, Sphalmioideae and Carnarvonioideae; taxa of Grevilleoideae are underlined and have wider branches.

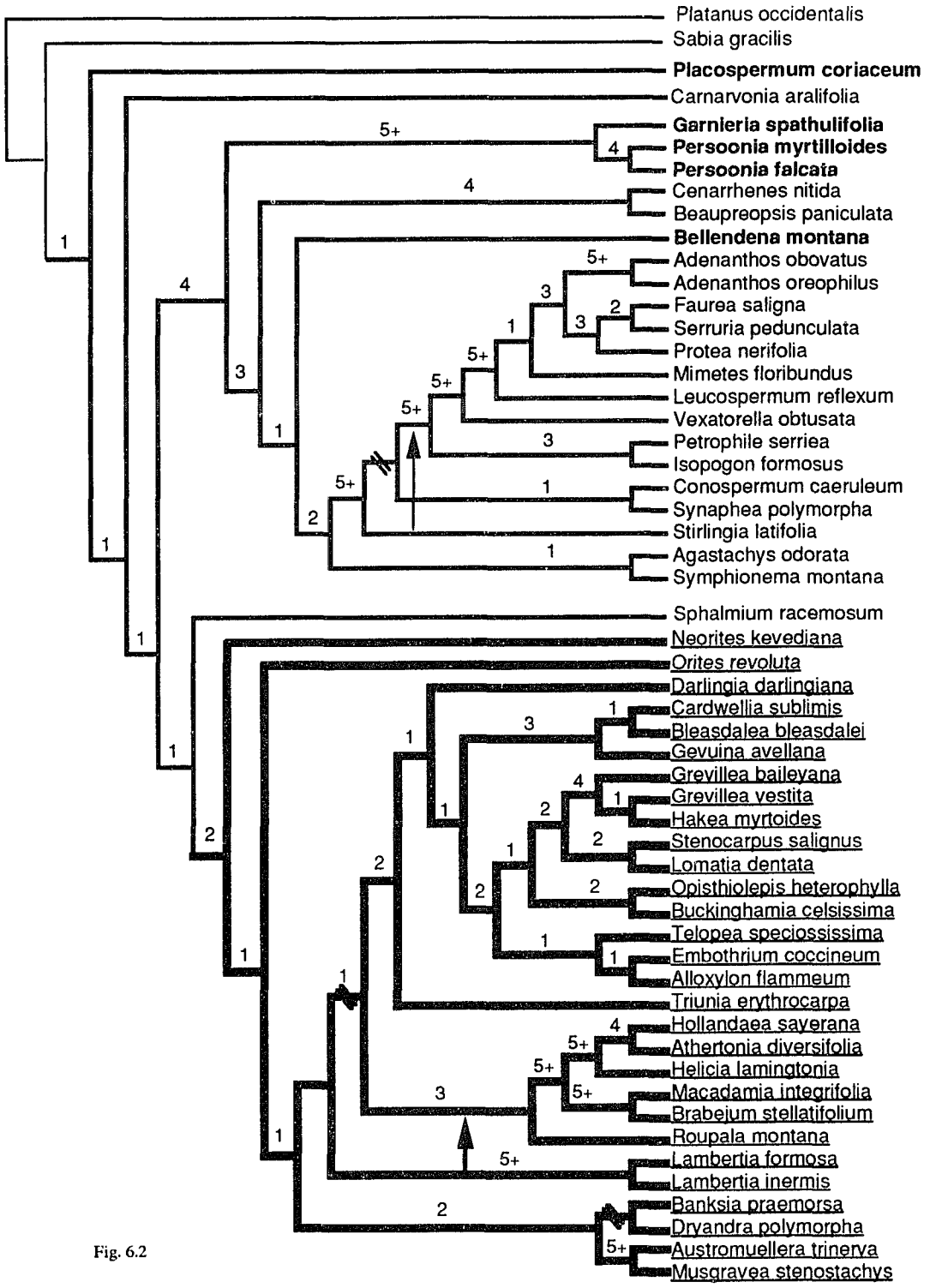

Platanus occidentalis
Sabia gracilis
Placospermum coriaceum
Carnarvonia aralifolia
Garnieria spathulifolia
Persoonia myrtilloides
Persoonia falcata
Cenarrhenes nitida
Beaupreopsis paniculata
Bellendena montana
Adenanthos obovatus
Adenanthos oreophilus
Faurea saligna
Serruria pedunculata
Protea nerifolia
Mimetes floribundus
Leucospermum reflexum
Vexatorella obtusata
Petrophile serriea
Isopogon formosus
Conospermum caeruleum
Synaphea polymorpha
Stirlingia latifolia
Agastachys odorata
Symphionema montana
Sphalmium racemosum
Neorites kevediana
Orites revoluta
Darlingia darlingiana
Cardwellia sublimis
Bleasdalea bleasdalei
Gevuina avellana
Grevillea baileyana
Grevillea vestita
Hakea myrtoides
Stenocarpus salignus
Lomatia dentata
Opisthiolepis heterophylla
Buckinghamia celsissima
Telopea speciossissima
Embothrium coccineum
Alloxylon flammeum
Triunia erythrocarpa
Hollandaea sayerana
Athertonia diversifolia
Helicia lamingtonia
Macadamia integrifolia
Brabejum stellatifolium
Roupala montana
Lambertia formosa
Lambertia inermis
Banksia praemorsa
Dryandra polymorpha
Austromuellera trinerva
Musgravea stenostachys

Fig. 6.2

"uncertain characters" that involve the flower-pair characters are only coded among Grevilleoideae: they are coded as "uncertain" among all other taxa. These characters are diverse among Grevilleoideae. They they did not affect the topology of clades outside of Grevilleoideae or the position of Grevilleoideae among other Proteaceae. Therefore, taxon and character distributions are discussed within the context of the one tree that has a similar topology to the majority rule consensus obtained from the second analysis except for Stirlingia. I selected the tree that resolved Stirlingia as the basal sister group to Conospermum/Synaphea based on the actinomorphic flower form and the presence of four fertile anthers in Stirlingia instead of the zygomorphic flowers with one fertile and two half-fertile, laterally suppressed, anthers in the latter taxa (Fig. 6.2).

To examine the stability of nodes in the cladogram, decay analysis (Bremer, 1988) techniques were employed (figures are listed below the nodes in bold face in Figure 2). There is little support for the Sphalmium/Grevilleoideae clade and the position of Carnarvonia (1 step). There is relatively strong support of 4 steps for the Proteoideae/Persooniinae clade and for the inclusion of Adenanthiinae in the Proteinae (Proteeae) clade of 3 steps.

DISCUSSION

Compared to other classifications and published phylogenies of Proteaceae, the most-parsimonious phylogeny presented here shares most similarities to the subfamilies of Johnson and Briggs (1975) except for Persoonioideae. Based on the phylogeny presented here, the family is primarily divided based on the fruit characters. As inferred by Johnson and Briggs (1975), the follicular fruit appears to represent the basal condition of Proteaceae while the drupaceous nuts and achenes are derived in the Persooniinae/Proteoideae clade. For purpose of discussion, one of the six trees that was obtained via the exclusion of the unknown characters (second analysis) is described. It

should be noted that the same tree topology is present in one of the 22 trees from the first analysis. The 21 other most-parsimonious arrangements obtained from the first analysis and the other five most-parsimonious arrangements from the second analysis (all of which had a same topology to five of the 22 trees in the first analysis) are equally plausible hypotheses. Reference is made to those instances of lack of resolution below.

Proteaceae appears monophyletic. However, the data provide strong support for a reclassification of Proteaceae at subfamilial, tribal and subtribal levels. Based on the proposed phylogeny (Fig. 6.2), the phylogenetic origin of specific floral and inflorescence characters can be examined. There are several hypotheses concerning the development, homology and diverse nature of the nectaries in Proteaceae (Chapter 2,3), the gynoecium orientations (Chapter 5) and the origin of the flower-pairs in Grevilleoideae (Chapter 4). Based on both ontogenetic comparisons and the principles of parsimony, the hypotheses surrounding the diverse character suites are compared.

Phylogenetic resolution among Proteaceae - Compared to the phylogeny proposed by Johnson and Briggs (1975) and the unstructured classification of Venkata Rao (1971), there is strong resolution of relationships among proteaceous taxa examined as determined from parsimony analysis (Fig. 6.2). In the proposed phylogeny of Johnson and Briggs (1975), there is little resolution among subfamilies except for Persoonioideae, as the basal proteaceous group and Carnarvonioideae as the basal taxon to Grevilleoideae. Other relationships in Johnson and Briggs (1975) are unresolved, including genera in subtribes, subtribes within tribes, and tribes within subfamilies. The differences between Johnson and Briggs (1975) phylogeny and the phylogeny obtained in this study are discussed below (Fig. 6.3). In the presented phylogeny, many of the taxa that Johnson and Briggs (1975) assigned different supra-generic rankings are not supported as monophyletic groups.

Are the subfamilies and other suprageneric taxa sensu Johnson and Briggs monophyletic? - Persoonioideae - Johnson and Briggs (1975) defined five subfamilies: 1. Persoonioideae; 2. Sphalmioideae; 3. Grevillioideae; 4. Proteoideae; and 5. Grevilleoideae. Persoonioideae was further divided into three subtribes in two tribes: Persooniinae (five genera, reduced to two genera by Weston, 1983) and Placosperminae (monotypic) in the Persoonieae and the monotypic Bellendena in tribe Bellendeneae. The primary character they used for the subfamilial status of Persoonioideae is the diploid condition of the chromosomes (n=7 in Persoonieae and n=5 in Bellendeneae). In all other Proteaceae, the taxa appear to be tetraploid or tetraploid derivatives (Johnson and Briggs, 1975).

There is no support for a monophyletic Persoonioideae from the present analysis. Placospermum represents the basal taxon to the family. Johnson and Briggs (1963, 1975) suggested that Placospermum is the "archaic taxon" of the family. Feuer (1986) supported the contention of Johnson and Briggs based on comparative pollen ultrastructure studies. The flowers of Placospermum are highly specialized in form, having three staminodia, a stigma on the abaxial side of the carpel, and zygomorphic, elongate, tubular red flowers. Another character that is unique to the taxon is that orthotropous ovules face the back of the locule and do not point down like most of the other proteaceous taxa with orthotropous ovules (also observed by Venkata Rao, 1971; Johnson and Briggs, 1975).

Strong support for Persooniinae and Bellendeneae as components of Proteoideae (sensu Johnson and Briggs, 1963) is inferred from decay analyses (+ 4 steps; Fig. 6.2). Persooniinae is monophyletic supported by several character states: centrifugally expanded capitate stigma, acutely tapered Vorläuferspitze, drupaceous fruit, and a sub-stipitate cushion as well as the architecture of the flowering branches (single flowers borne

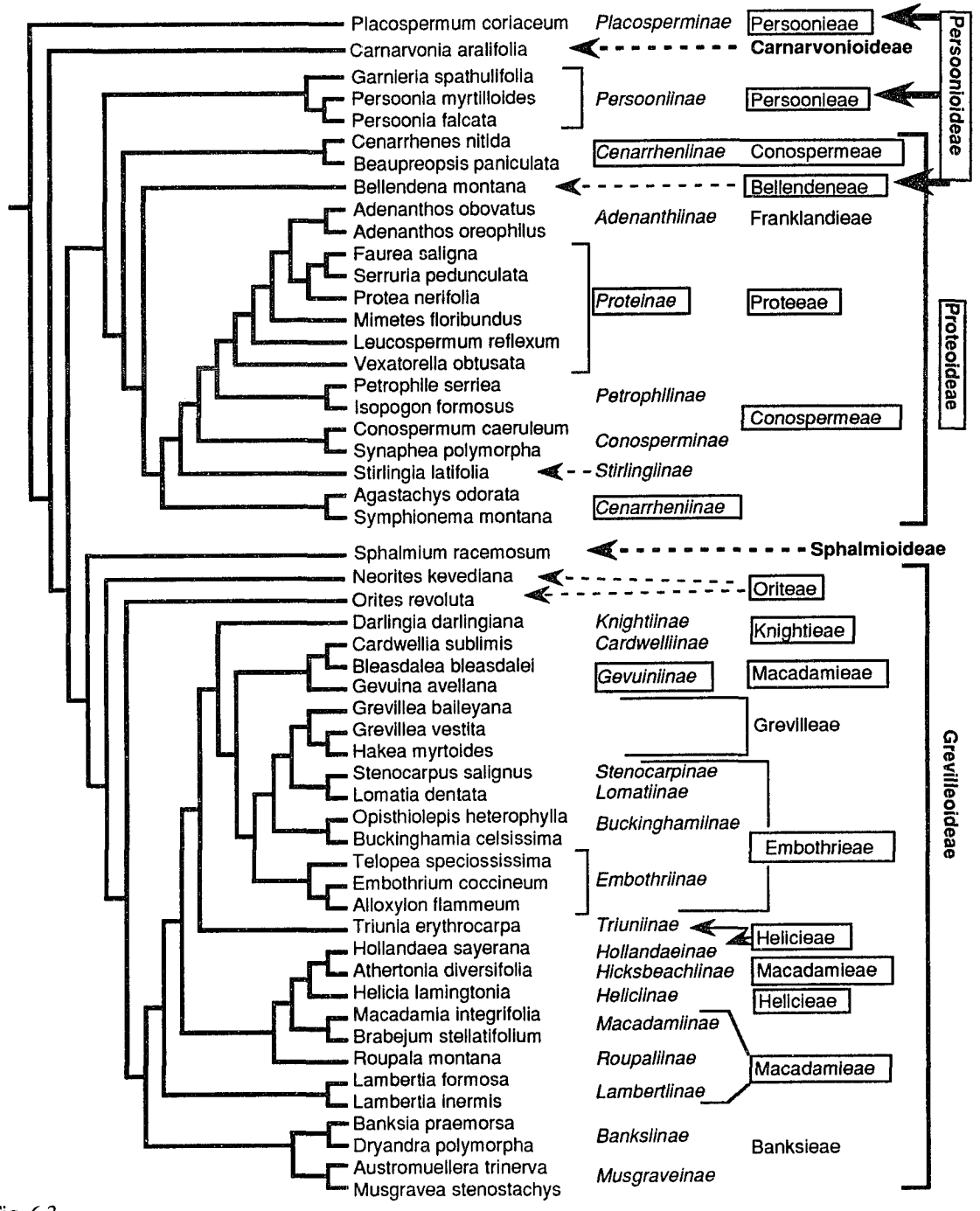
in leaf axils along anauxotelic shoots; Weston and Johnson, 1991) in the genus Persoonia. Bellendena appears to represent a taxon basal to the morphologically diverse Proteoideae. In particular Bellendena is sister taxon to Agastachys/Symphionema. Venkata Rao (1971) proposed that Bellendena characterizes the basal proteaceous taxon based on the premise that the flowers represent the primitive, generalized form compared to other proteaceous taxa; the flowers have free filaments, generally unspecialized anthers, four free and relatively unmodified tepals, no nectariferous glands, a single tepal trace that splits into three, and a 1n chromosome number of five. He further asserted that Symphionema (n=10, single traced) and Agastachys (n=11, single traced) represent the extant early off-shoots of a Bellendena-like ancestor. The other Proteoideae (sensu Venkata Rao, 1971 that includes Persoonioideae) were subsequently derived from such taxa. The hypothesis that Bellendena represents the basal proteaceous taxon (sensu Venkata Rao, 1971) is not supported although Bellendena represents the sister group to Agastachys/Symphionema and a proliferative grade of Proteoideae (sensu Johnson and Briggs, 1975).

Proteoideae, as defined by Johnson and Briggs (1975), is not monophyletic in this analysis. Bellendena (Persoonioideae) has a supra-basal node within Proteoideae, specifically within the tribe Conospermeae and subtribe Cenarrheninae (Fig. 6.3). Johnson and Briggs (1975) segregated Proteoideae into three tribes and nine subtribes: **Conospermeae** - Dilobeinae (not examined); Cenarrheninae, Stirlingiinae, Conosperminae, and Petrophilinae;

Franklandieae - Franklandiinae (not examined) and Adenanthiinae; and **Proteeae** - Aulacinae (not examined) and Proteinae (Fig. 6.3). These will be discussed individually.

Conospermeae (sensu Johnson and Briggs, 1975) does not constitute a monophyletic group (Fig. 6.3); rather it represents a para- and polyphyletic grade. Each

Figure 6.3. One of the most-parsimonious trees obtained from all three analyses (see text and Fig. 6.2) compared to Johnson and Briggs's (1975) phylogeny. Subfamilies are bracketed on the right; the second row from the right lists the tribes; the third row from the right, next to the species, lists the subtribes. Supra-generic rankings enclosed by boxes represent non-monophyletic groups compared to this analysis.


Placospermum coriaceum
Carnarvonia aralifolia
Garnieria spathulifolia
Persoonia myrtilloides
Persoonia falcata
Cenarrhenes nitida
Beaupreopsis paniculata
Bellendena montana
Adenanthos obovatus
Adenanthos oreophilus
Faurea saligna
Serruria pedunculata
Protea nerifolia
Mimetes floribundus
Leucospermum reflexum
Vexatorella obtusata
Petrophile serriea
Isopogon formosus
Conospermum caeruleum
Synaphea polymorpha
Stirlingia latifolia
Agastachys odorata
Symphionema montana
Sphalmium racemosum
Neorites kevedlana
Orites revoluta
Darlingia darlingiana
Cardwellia sublimis
Bleasdalea bleasdalei
Gevuina avellana
Grevillea baileyana
Grevillea vestita
Hakea myrtoides
Stenocarpus salignus
Lomatia dentata
Opisthiolepis heterophylla
Buckinghamia celsissima
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Roupala montana
Lambertia formosa
Lambertia inermis
Banksia praemorsa
Dryandra polymorpha
Austromuellera trinerva
Musgravea stenostachys
Placosperminae
Persoonieae
Carnarvonioideae
Persoonlinae
Persoonieae
Cenarrhenlinae
Conospermeae
Bellendeneae
Adenanthlinae
Franklandieae
Proteinae
Proteeae
Petrophilinae
Conospermeae
Conosperminae
Stirlinglinae
Cenarrheniinae
Sphalmioideae
Oriteae
Knightiinae
Cardwelliinae
Knightieae
Gevuiniinae
Macadamieae
Grevilleae
Stenocarpinae
Lomatiinae
Buckinghamiinae
Embothrieae
Embothriinae
Triuniinae
Helicieae
Hollandaeinae
Hicksbeachiinae
Macadamieae
Heliciinae
Helicieae
Macadamiinae
Roupaliinae
Lambertiinae
Macadamieae
Bankslinae
Banksieae
Musgraveinae
Persoonioideae
Proteoideae
Grevilleoideae

Fig. 6.3

subtribe in Conospermeae is supported as a monophyletic group except for Cenarrheninae. The Cenarrhenes/Beaupreopsis branch is monophyletic but is separated from the other taxa by appendicular connectives (synapomorphy) and the spatulate anther connective (a synapomorphy). Other features of the taxa are shared (symplesiomorphies) with the basal taxa of Persooniinae include: axillary inflorescences, the presence of wide nectaries (pileate shape of the nectary lobes is a synapomorphy), and tardily fleshy drupes. Some of the characters that support the clade of Proteoideae excluding Cenarrhenes/Beaupreopsis include the synapomorphies terminal inflorescence, single seeded dry fruit, glandless flowers, no appendicular connective, two orthotropous ovules (one is aborted), and lack of a pedicel (excluding Bellendena).

The African subtribe **Proteinae** (sensu Johnson and Briggs, 1975) is not monophyletic. The Australian subtribe **Adenanthiinae**, with the single genus Adenanthos, is immersed within Proteinae. It should be noted that material of the other subtribes of Proteineae and Adenanthiinae were not available for incorporation in this analysis. Nelson (1978) challenged the exclusion of Adenanthos from other Proteinae in Johnson and Briggs (1975), and suggested that Adenanthos is more closely related to Mimetes and Spatalla (not examined here) than it is to Franklandia (not examined). Venkata Rao (1971) defined the tribe Proteeae to include Adenanthos as well as the Australian Petrophilinae. Petrophilinae represents the sister clade to Proteeae and Adenanthiinae. Character states that support the inclusion of Adenanthiinae in Proteinae include: reduced axillary inflorescences, callus structures on the leaf tips, four laminar nectariferous lobes on a short hypanthium, fruit and seed characters (sensu Manning and Brits, 1993), modified stylar outgrowths or pollen-presenter (derived in Petrophilinae, the sister clade from Conospermeae), a subterminal stigma that remains closed until receptivity, and abaxial curvature of the style in bud. The latter character is shared with Faurea, Serruria and Protea. Jordaan (1944) and more recently, Rourke (in Dyer, 1975;

Rourke, 1984a,b) and Midgley (1987) contend that there is a distinct group of South African Proteinae called the Leucospermum group that excludes Protea and Faurea. Such a group does not appear monophyletic, based on the present analysis, although seven genera of Proteeae are not included in the present analysis. Interestingly, Rourke (1984b) suggested that Vexatorella could represent the "archaic taxon" to the African Proteoideae, a contention that is supported by this parsimony analysis. Seed and fruit characters have proven vital in determining specific relationships among genera of Proteaee (Rourke, 1972, 1984a,b; Midgley, 1987), but are not included in this phylogenetic analysis. Although there are numerous anatomical investigations of seeds and fruits available in the literature, a recent review by Manning and Brits (1993) provides detailed accounts of the misinterpretations in virtually all of the fruit and seed literature for Proteaceae including Venkata Rao, 1971 and Johnson and Briggs, 1975. A primary misinterpretation of proteaceous fruits involves the interpretation of the crystalliferous endocarp in some taxa, compared with the crystalliferous tegmen and endotesta of other taxa (sensu Venkata Rao, 1971). In fact, the crystalliferous layer in all investigated Proteaceae is the endotesta (inner epidermis of the outer integument) and not the endocarp (Manning and Brits, 1993).

Carnarvonioideae is composed of two species in N.E. Queensland rainforests. Both Venkata Rao (1971) and Johnson and Briggs (1975) contended that Carnarvonia represents a transitional or intermediate to Grevilleoideae, primarily based on the inflorescence which is short, four- to eight- flowered heterothetic and frondobracteose, and racemoid. The flowers are unique and actually share a number of features (symplesiomorphic characters) with those in Persooniinae (papillate trichomes on the inner epidermis of the tepals, cushion at base of ovary; broadened tepal claws; short Vorläuferspitze) and with Bellendeneae (lacking a nectary, punctiform stigma, and a short stipe). The leaves are compoundly palmate, an autapomorphy to the taxon. Although the

fruit are dehiscent follicles containing winged seeds, the follicle is unique and resembles a tapered and asymmetrical flask (pers. obs.). From this analysis, Carnarvonioideae is an early offshoot of Proteaceae and represents a taxon that has had a long and isolated history. Additional developmental and morphological analysis of this taxon is necessary prior to a discussion of its unique features and phylogenetic relationships.

Sphalmioideae is another monotypic N.E. Queensland endemic described in 1975 by Briggs, Hyland and Johnson. Johnson and Briggs (1975) and Briggs, Hyland and Johnson (1975) inferred that Sphalmioideae, like Carnarvonioideae, was derived early in the history of the family and has had a "not very successful evolutionary history." They also claim that Sphalmioideae probably diverged somewhere near the base of the "line" (quotes in Johnson and Briggs, 1975) that led to Grevilleoideae. In the present analysis, Sphalmium is the basal taxon of a monophyletic Grevilleoideae. There are a number of similarities in the nature of the follicular fruit (pers. obs.; Johnson and Briggs, 1975), wood anatomy (Chattaway, 1948), and pollen characters (Feuer, 1986). Character states that support its position as a basal member to Grevilleoideae include the almost free or loosely adnate filaments, axillary inflorescences, secondary and tertiary venation patterns, clavate almost decurrent stigma or suture, short style, the absence of a pollen presenter (a plesiomorphy?), short, hirsute pedicels, a densely hirsute ovary and hemitropous almost anatropous ovules as found in Neorites. Interestingly, Sphalmium was first described as a new species of Orites, based on similar features to the N.E. Queensland Orites. Additional developmental and morphological analysis of Sphalmium is necessary prior to a detailed discussion of other morphological features.

Grevilleoideae is the largest subfamily with 41 genera. In this analysis, Grevilleoideae (sensu Johnson and Briggs, 1975) is monophyletic. A single unifying character of the subfamily is the presence of paired flowers, defined as two-flowered short-shoots (Douglas, Chapter 4). The subfamily is divided into seven tribes by Johnson

and Briggs (1975; Fig. 6.3) and eight tribes by Venkata Rao (1971). In most cases, the tribes and subtribes are not monophyletic as discussed below.

Oriteae - Tribe Oriteae is composed of two genera: the monotypic, multiovulate Neorites from N.E. Queensland and the biogeographically and morphologically diverse biovulate Orites with species in E. Queensland, S.E. Victoria and N.S.W., Tasmania and Chile. Only Orites revoluta was available for inclusion in this analysis. The tribe does not appear to be monophyletic and represents a paraphyletic grade leading to other Grevilleoideae. There are a number of dramatic differences between the two genera. Neorites, like Sphalmium, lacks a pollen presenter and has a carpel that is shorter than the anthers. In Orites revoluta the pollen from the anthers is deposited onto the upper portion of the elongate style, that functions as a pollen-presenter without morphological differentiation of the style. Pollen presentation on the style is a synapomorphy for Orites and all other Grevilleoideae. It should be noted that there are successive modifications of the style directly involved with pollen presentation in other Grevilleoideae. Other species of the Queensland Orites (namely Orites excelsa) do not present the pollen on the style (Johnson and Briggs, 1975), and the carpel resembles those in Sphalmium and Neorites.

Banksieae is composed of two subtribes sensu Venkata Rao (1971) and Johnson and Briggs (1975): the N.E. Queensland Musgraveinae, including the monotypic Musgravea stenostachya and the two species of Austromuellera, and the virtually pan-Australian speciose genera of Banksiinae including Banksia and Dryandra. Banksieae (sensu Johnson and Briggs, 1975) is supported as a monophyletic tribe in this analysis. Synapomorphies to the clade include the character states of biporate, ellipsoidal pollen grains, deeply lobed pinnatisect leaves (compoundly pinnate in Austromuellera) and tertiary orthogonal reticulation. The subtribe Banksiinae was equally resolved as a monophyletic and a paraphyletic group in the most-parsimonious trees of all three analyses (Figure 2). The lack of resolve could be due to taxon sampling. Interestingly though, a

recent phylogenetic analysis of Banksiinae (P. Ladiges, pers. comm.) hypothesizes that Banksia is a paraphyletic group around the monophyletic Dryandra.

Macadamieae is biogeographically diverse and is described as a heterogeneous group of 15 genera in six subtribes, primarily distinguished from other grevilleoids by the presence of two "more-or-less" pendulous ovules (quotes in Johnson and Briggs, 1975). Macadamieae is not monophyletic in the present analysis, although a number of the subtribes (sensu Johnson and Briggs, 1975) are monophyletic. Lambertiinae, a unigeneric subtribe of Macadamieae, is parsimoniously placed either as the sister clade to Banksieae (Fig. 6.3) or as the basal clade of the Roupala clade (Fig. 6.2). Venkata Rao (1971) assigned tribal status to Lambertia based on its peculiar capitate inflorescences (Douglas, Chapter 4). The carpel orientation is unique in Lambertiinae (the cleft facing the margins of the abaxial and lateral tepal closest to the common bract; Fig. 6.7).

Other subtribes of Macadamiinae (sensu Johnson and Briggs, 1975) are not monophyletic. **Roupaliinae** has two genera, of which only the tropical American Roupala was available for analysis; the other genus is the monotypic New Caledonian Kermadecia. Roupala represents the basal member of the dichotomous clade that contains the monophyletic **Macadamiinae** (composed of the Australian Macadamia, the South American Panopsis, not examined, and the South African Brabejum). Macadamiinae is the sister taxon to a clade of taxa from two different tribes: **Helicieae** including Helicia and Hollandaea, and the macadamiean subtribe **Hicksbeachiinae,** represented here by Athertonia. Synapomorphies for the Roupaliinae/Macadamiinae/Helicieae/Hicksbeachiinae clade include tardily dehiscent to drupaceous fruits, non-winged seeds, a four-lobed nectary with intercalary growth between the lobes, and an ellipsoidal, club-shaped pollen-presenter.

Gevuiniinae (Macadamieae) contains four genera of which only the South American Gevuina and the Australian/Malesian Turrillia (syn. Bleasdalea) are included; the other two genera are the tropical american Euplassa and the New Caledonian Sleumerodendron. Gevuina and Bleasdalea: are more closely related to Cardwellia of the Knightieae in the present analysis. There are a number of characters that support the position of Gevuina and Bleasdalea namely, the common peduncle and lack of pedicels, multi-papillate hairs on the pollen-presenter prior to anthesis, and the ventral-dorsally oriented carpels in Cardwellia and Bleasdalea. **Floydiinae** contains the monotypic Australian Floydia, which was not available for this study.

Helicieae is divided into three subtribes: the monotypic Hollandaeinae; the digeneric Heliciinae including the speciose Malesian genus Helicia (material of Xylomelum was not available); and the monotypic Triuniinae (Triunia erythrocarpa). Helicieae is not a monophyletic tribe in this analysis. Triunia is sister taxon to Darlingia and together, the two taxa form a grade leading to the tribes of Knightieae, Grevilleae, and Embothrieae. Triunia erythrocarpa is an unusual northern Queensland rainforest understory tree with bright red, drupaceous fruits that are extremely toxic to humans following ingestion.

Knightieae is composed of two subtribes: the monotypic Cardwellinae and Knightiinae which includes three genera: Knightia with two species in New Caledonia and New Zealand, Eucarpha with two species in New Caledonia, and Darlingia with two species in N.E. Queensland. Included in this analysis are Cardwellia sublimis and Darlingia darlingiana. The tribe does not appear to be monophyletic. Darlingia is the basal taxon to the Cardwellia clade and the grade of Embothrieae and Stenocarpinae (Fig. 6.3). Darlingia has a terminal stigma that is surrounded by a ring of papillate cells, a synapomorphy for the more derived grevilleoid clades. The flowers of Darlingia are plesiomorphically actinomorphic at anthesis with fully reflexing and recurving tepals; the

more derived taxa are highly zygomorphic, having tepals of different lengths, arched styles, bilateral to asymmetrical floral receptacles, and morphologically diverse nectary lobes, not four lobes like Darlingia and the more basal Grevilleoideae. Cardwelliinae shares the ventral-dorsal orientation of the carpel, elongate peduncle, lack of pedicels, abaxial curvature of the perianth, erect adaxial lobe at anthesis, and four broad, pileate nectariferous glands with Bleasdalea, Gevuina (except for the carpel orientation), and Sleumerodendron (not included). In addition, the ovules of Cardwellia are tangentially displaced in the locule after initiation, a character shared with Bleasdalea.

Embothrieae and Grevilleae form a monophyletic group. Embothrieae is a paraphyletic assemblage or grade of monophyletic subtribes (sensu Johnson and Briggs,1975), that are basal to the monophyletic Grevilleae. Synapomorphies supporting the Embothrieae/Grevilleae clade include seed wings, usually multiovulate carpels (reduced to two in Grevilleae) a phalliform or discoid pollen-presenter, elongate stipes, elongate pedicels, hemitropous ovules, no elongation of the peduncle subtending the flower pair, highly zygomorphic flowers, reduced nectary lobe number, hippocrepiform-shaped nectary, and virtually complete loss of stamen filaments. Two developmental characters that support the Embothrieae/Grevilleae clade include the lateral initiation of the carpel and the reduction or complete loss of individual floral bracts in Grevilleae and Embothriinae.

Embothriinae (four genera; 11 species sensu Crisp and Weston, 1987) is the basal clade to Buckinghamiinae (two genera; three species) which is basal to a clade of Stenocarpiinae (two genera; 30 species) and Lomatiinae (unigeneric; 12 species). Venkata Rao (1971) suggested that the Chilean Embothrium (with biporate pollen like some Chilean Oreocallis, Feuer, 1989, 1990) should be classified as a separate tribe excluding the Australian taxa, Telopea and Alloxylon. Based on the present analysis and the analyses of Crisp and Weston, (1987; unpub.), Embothrium should remain a member of

the monophyletic Embothriinae. Synapomorphies among taxa of Embothriinae include the character states of a ring of hairs around the porate stigma, diagonally oriented carpel, and tertiary venation patterns in the leaf.

Buckinghamiinae is monophyletic in the present analysis. Feuer (1990) claims that there are few pollen characters uniting the two genera (Buckinghamia and Opisthiolepis). The flowers and inflorescences of the two taxa also appear morphologically unlike and unique. However, developmental studies indicate that the taxa share some unique character states relative to other Embothriinae, including the initiation of two nectary lobes followed by intercalary growth between the two lobes, terminal inflorescences (sympodial growth), broadly cuneate common bracts, elongate and free funiculi, and leaf vascular characters.

Lomatiinae, a unigeneric subtribe is classified as the sister taxon to Embothriinae in Johnson and Briggs (1975) and is the sole outgroup to Embothriinae by Crisp and Weston (1987). In the present analysis, Lomatiinae is the sister taxon to **Stenocarpiinae**. These two subtribes form a dichotomous clade basal to Grevilleae. Feuer (1990) asserts that Lomatia has very distant ties to Embothriinae based on ultrastructural pollen features. She claims that the pollen of Lomatia is very similar to the pollen of Stenocarpus,by the presence of several unifying features including columellate exine and a feature common to Lomatia and Stenocarpus: irregularly evaginate, microperforate, muri.

The sister clade to the Stenocarpiinae/Lomatiinae clade is the tribe **Grevilleae** with three genera, of which only two were included here. It should be noted that Grevillea appears paraphyletic in this analysis, a probable consequence of taxon sampling.

Floral diversity and evolutionary trends - Innate to a phylogenetic hypothesis of taxa are transformations of morphological character changes

and innovative events that have resulted in the observed morphological diversity of features. After all, floral diversity is essentially a reflection of the multifarious interactions of floral developmental processes. Combined with comparative ontogenetic analyses, the hypothetical transformations can be examined using methods of parsimony, and their ontogenetic and phylogenetic likelihood evaluated. This methodology has proven to be valuable in phylogenetic studies of different angiosperm groups. For example, In Piperales (Tucker, Douglas and Liang, 1993), ontogenetic characters and transformations were included in a phylogenetic analysis. In that analysis, parsimony methods implied two most-parsimonious trees. Using evidence of carpel development, one most parsimonious hypothesis is argued as developmentally unlikely based on a reversal from a synorganized flower with a syncarpous gynoecium to the reverse condition of apocarpy. Developmental likelihood suggests that such a transformation would be improbable in Saururaceae.

In the following sections, I compare some of the morphological features examined from separate ontogenetic analysis of proteaceous flowers, and discuss the phylogenetic implications. Characters included here are primarily floral and inflorescence features with enigmatic homology, including the nectaries, the carpel, and the inflorescence and flower pairs.

Nectaries - The presence of a single perianth whorl and the general presence of a four-lobed, alternitepalous nectary among proteaceous taxa has probably been the single factor most confounding to the establishment of affinities of Proteaceae to other angiosperms. In all taxa, the nectary develops relatively late in development, after the organs have been initiated and as the receptacle is broadening (Douglas, Chapter 2, 3). Differing interpretations of the homology of the nectary include: reduced petals (Haber, 1959, 1961, 1966; Eames, 1961); a derived functional enation from a basically glandless ancestor (Venkata Rao, 1971); or a basic or general enation present among the early

ancestors of the family, that has subsequently become morphologically and taxonomically diverse (Johnson and Briggs, 1975). The interpretation that the nectary represents vestigial petals is developmentally unfounded (Douglas, Chapter 2) and phylogenetically non-parsimonious. The nectary is initiated inside of the stamen whorl and relatively late in the development of the flower. The closest outgroup to Proteaceae (sensu Chase et al., 1993) is Sabia, that also has a nectary in addition to two perianth whorls. Using phylogenetic evidence, the nectary appears to have been a component of taxa in the early diversification of the family, contrary to Venkata Rao's hypothesis. Considering the phylogenetic and developmental evidence, the nectary is a functional enation, not homologous to any reduced primary organs in Proteaceae. The nectary was most likely present early in the diversification of Proteaceae, as hypothesized by Johnson and Briggs (1975).

Within the family the form, shape and phylogeny of the nectary poses a number of interesting problems. A primary problem is the apparent loss followed by the gain of the nectary in the clade containing Persooniinae, Bellendeneae and Proteoideae (character tree in Fig. 6.4). The lack of a nectary was used to define the tribe Conospermeae (Johnson and Briggs, 1975). Venkata Rao (1971) similarly used the lack of a nectary as the primitive condition in Bellendena and Symphionema/Agastachys, and asserted that the nectary is derived in other Proteaceae. The nectary in Persoonieae (Fig 4A) and in the clade of Cenarrhenes/Beaupreopsis (Fig. 6.4B) is composed of four free alternitepalous lobes. There is a loss of the nectary in Bellendena (Fig. 6.4C) that is maintained for several nodes in the tree, followed by the regaining of the nectary in a similar although more laminar form among Proteinae/Adenanthiinae (Figs. 6.4D-E). The most parsimonious interpretation of this phenomenon in this analysis is two steps, from a plesiomorphic presence to a derived loss and then to a derived regaining of the nectary. Developmentally, the reappearance of a "lost structure" will seldom resemble the original

Figure 6.4. Distribution of nectaries types of Proteoideae and Persooniodeae on partial cladogram. The distribution suggests that the nectaries are a basal component of the family (narrow lines) that were lost in some taxa, particularly the grade of Conospermeae and Bellendena (thick lines) and then secondarily regained or derived in Proteeae and Adenanthiinae (hatched lines). Micrographs of nectaries. **4A**. Nectary morphology among Persooniinae (Persoonia myrtilloides). **4B**. Morphologically divergent nectary in Cenarrhenes nitida. **4C**. Receptacle, stipe, and carpel base of Bellendena montana. No nectaries are produced. **4D**. Nectary initiation in Serruria pedunculata. The nectary is initiated from a short hypanthium. **4E**. Mature nectary form in Serruria pedunculata is thin and laminar. **4F**. Nectary form in Neorites kevediana as a representative of Grevilleoideae. Scale bars = 500μm.

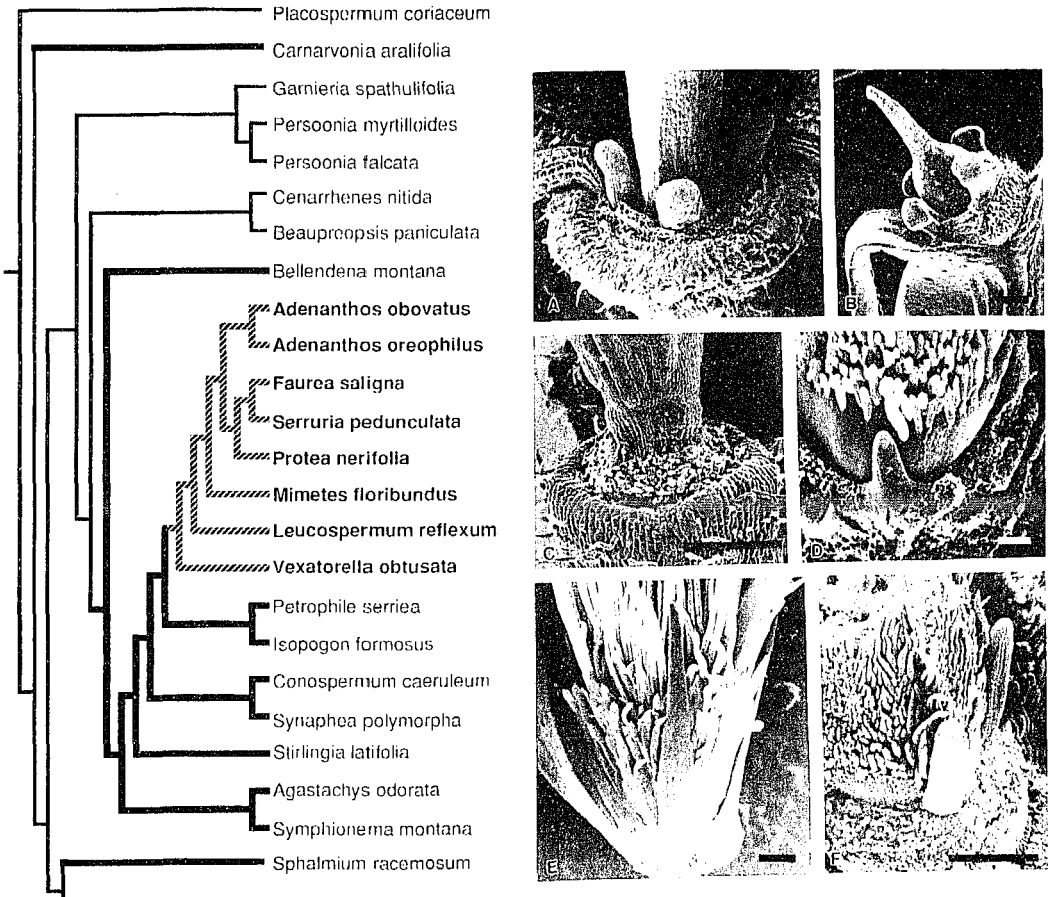
Placospermum coriaceum
Carnarvonia aralifolia
Garnieria spathulifolia
Persoonia myrtilloides
Persoonia falcata
Cenarrhenes nitida
Beauprcopsis paniculata
Bellendena montana
Adenanthos obovatus
Adenanthos oreophilus
Faurea saligna
Serruria pedunculata
Protea nerifolia
Mimetes floribundus
Leucospermum reflexum
Vexatorella obtusata
Petrophile serriea
Isopogon formosus
Conospermum caeruleum
Synaphea polymorpha
Stirlingia latifolia
Agastachys odorata
Symphionema montana
Sphalmium racemosum
GREVILLEOIDEAE
A
B
C
D
E
F

FIG. 6.4

form (Gould, 1977; Tucker, 1988); therefore, one would expect some anatomical, morphological or developmental differences between the nectary in the basal taxa and the derived nectary in the Proteinae/Adenanthiinae clade. The lobes of the nectary are vascularized by the floral stele in Cennarhenes/Beaupreopsis and by the staminal traces in Persooniinae (Venkata Rao, 1967). In Proteinae, there is no vascularization of the nectary and in *some* species of Adenanthos the nectary vasculature originates from the lateral tepal traces (Venkata Rao, 1957, 1967b, 1971).

Developmentally, there are also differences. In taxa of Persooniinae, as well as in Grevilleoideae and Placospermum, the nectary lobes are initiated from the flat floral receptacle adjacent to the carpel and develop as broad lobes, becoming laminar and relatively thick in Placospermum and Persooniinae (Chapter 2). In Adenanthos, Serruria (Chapter 3), and Leucospermum, Vexatorella and Orothamnus (pers. obs.) the nectary lobes are initiated from the inner surface of a concave, short hypanthium and continue to develop as highly attenuate and thin laminar structures. Such evidence supports the contention that the nectary was lost in the line leading to Proteinae/Adenanthiinae and then regained. Detailed histogenetic investigations should reveal additional differences.

The diversity of nectary morphologies is extreme among Grevilleoideae. In its basic condition, the nectary is four-lobed as in Oriteae (Neorites in Fig. 6.4F), Banksiinae, Helicia, Triunia, Athertonia, Darlingia and Cardwellia. In other taxa, there can be loss of a single lobe, loss of two lobes, or the presence of intercalary growth between lobes, or the apparent lack of lobes on a hippocrepiform structure. There has been an apparent loss of the lobe on the dorsal side of the carpel among Musgraveinae and Lomatiinae. Intercalary growth between four lobes occurs among taxa of Lambertiinae, Macadamiinae and Hollandaea. Reduction to two lobes occurs in Gevuina and Bleasdalea, although in both taxa, all four lobes are initiated, the abaxial pair prior to the

adaxial pair. In Gevuina and Bleasdalea, the two adaxial lobes are suppressed following initiation and do not enlarge. Interestingly, in Cardwellia the abaxial pair of nectary lobes is initiated prior to the adaxial pair, although all four will enlarge equally.

Among Embothrieae, Stenocarpus and Grevilleae, a single hippocrepiform (crescent-shaped) nectary utilizes the remains of the floral apex, next to the ventral side of the carpel. In Buckinghamia and Opisthiolepis, two glands are initiated in this position and then intercalary growth between the two lobes produces a semi-circular disk or bilobed nectary. It should be noted that in Opisthiolepis, the two lobes elongate and become tapered. In Embothrium the hippocrepiform nectary becomes epigynous, attached to the elongating stipe. Venkata Rao (1971) and Johnson and Briggs (1975) assert that the diverse nectary morphologies represent derived features that are associated with the expression of zygomorphy among grevilleoid flowers.

Gynoecium diversity - Features associated with the carpels among Proteaceae are both taxonomically and morphologically diverse. The carpel is probably the most diverse floral organ in Proteaceae. Developmentally, there are different orientations of the carpel that are specific to genera among Grevilleoideae. The carpel initiaties terminally in most taxa, andlaterally in Grevilleae, Embothriinae, and two other genera of Embothrieae, Buckinghamia and Stenocarpus (Douglas, Chapter 5).

Carpel orientations (Fig. 6.5) - All proteaceous flowers have a single carpel. Among four of the five subfamilies, the carpel is the product of conversion of the entire floral apex after stamen initiation. In the fifth, the carpel is initiated laterally. The cleft faces the adaxial side of the flower (Fig. 6.5a). In taxa of Grevilleoideae the carpel cleft is oriented in different, generically conserved positions (Fig. 6.5b). The six orientations identified among different genera include; the carpel cleft faces the adaxial

Figure 6.5. Distribution of carpel orientations among Proteaceae (primarily Grevilleoideae) including the type of carpel initiation as terminal (outlined carpels) or lateral (black carpels and thick black branches). Variable branch lengths are for aesthetics and do not represent phylogenetic distances. The floral diagrams illustrate the four tepals arranged dorsiventrally, two in the median frontal plane and two in the median sagittal plane; the four stamens in superposed or antetepalous positions; and the carpel orientation is depicted by the cleft. The flowers are oriented in pairs in a common bract axil among all Grevilleoideae. These are two-flowered short-shoots, as each flower is subtended by a generally reduced floral bract. Symbols: PI = principal inflorescence axis; CB = common bract or first order pherophyll.

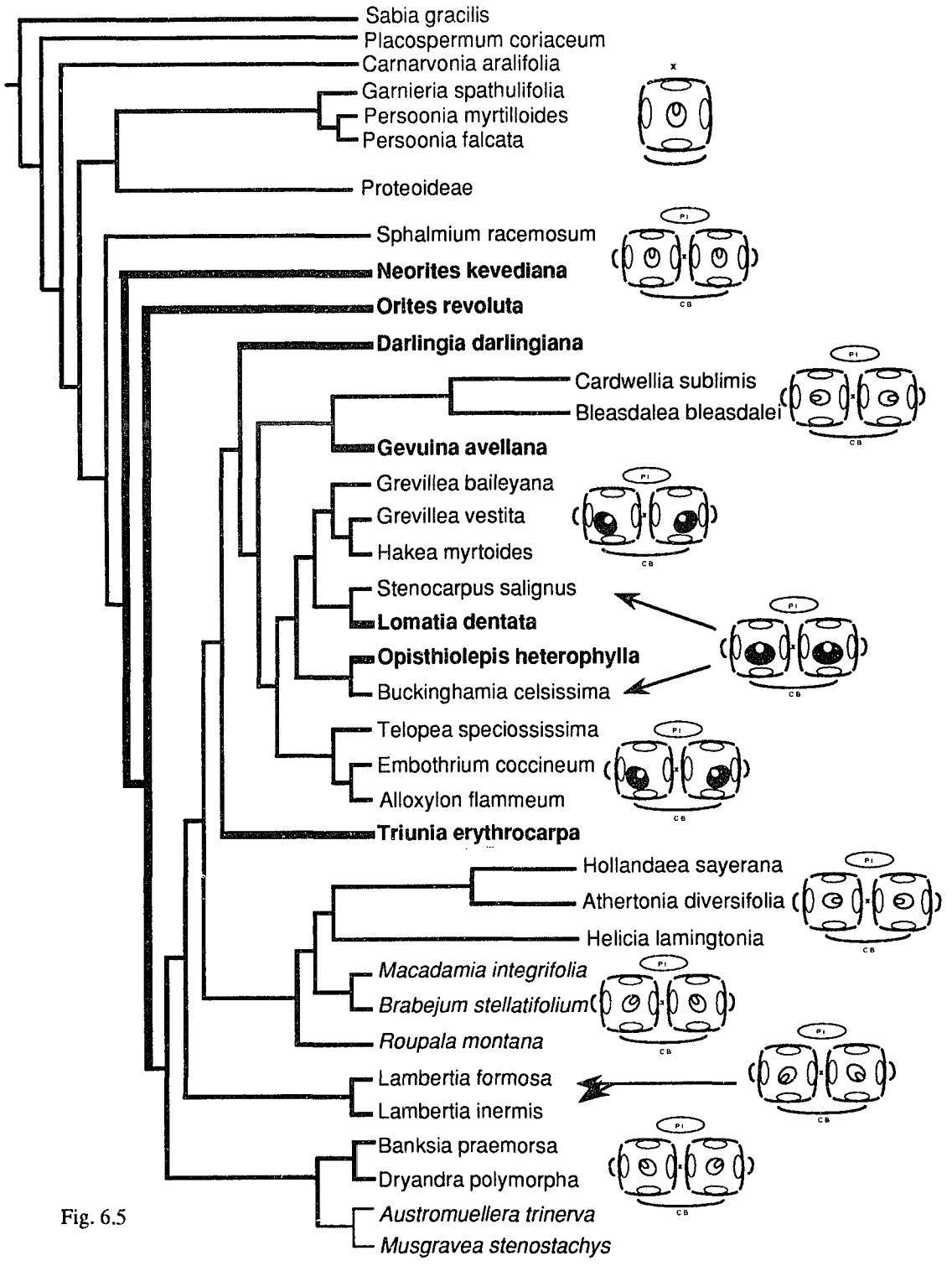
Sabia gracilis
Placospermum coriaceum
Carnarvonia aralifolia
Garnieria spathulifolia
Persoonia myrtilloides
Persoonia falcata
Proteoideae
Sphalmium racemosum
Neorites kevediana
Orites revoluta
Darlingia darlingiana
Cardwellia sublimis
Bleasdalea bleasdalei
Gevuina avellana
Grevillea baileyana
Grevillea vestita
Hakea myrtoides
Stenocarpus salignus
Lomatia dentata
Opisthiolepis heterophylla
Buckinghamia celsissima
Telopea speciossissima
Embothrium coccineum
Alloxylon flammeum
Triunia erythrocarpa
Hollandaea sayerana
Athertonia diversifolia
Helicia lamingtonia
Macadamia integrifolia
Brabejum stellatifolium
Roupala montana
Lambertia formosa
Lambertia inermis
Banksia praemorsa
Dryandra polymorpha
Austromuellera trinerva
Musgravea stenostachys

Fig. 6.5

tepal (Fig. 6.5c), the lateral tepal (Fig. 6.5d), the abaxial tepal (Fig. 6.5e), between the adaxial/lateral tepal (Fig. 6.5f), the abaxial/lateral tepal away from the primary inflorescence axis (Fig. 6.5g) and the abaxial/lateral tepal closest to the primary inflorescence axis (Fig. 6.5h).

Douglas (Chapter 5) defined the ontogenetic patterns of carpel organogenesis and reviewed possible events responsible for the diversity of orientations. Two hypotheses were offered, both of which can be addressed within a phylogenetic framework. In the first hypothesis, it is asserted that the diverse orientations are the product of different reduction events from a tetracarpellate ancestor (Venkata Rao, 1971). For example, the laterally-oriented-carpellate taxa could be derived from a tetracarpellate hypothetical taxon via loss of the sagittal carpels and one of the lateral carpels. As a corollary, the taxa with an adaxially facing carpel were presumably derived from a similar taxon via loss of the frontal and adaxial carpels. Basic to such a hypothesis is that Grevilleoideae would be non-monophyletic; something for which there is no evidence from the present phylogenetic analysis. In addition, developmental investigations demonstrate that there is no evidence of vestigial additional carpellate structures at the time of initiation.

In the second hypothesis (Douglas, Chapter 5), it is contended that the orientation of the carpel was not a conserved or developmentally constrained feature among early Grevilleoideae. Douglas (Chapter 5) adds that the orientation of the carpel became secondarily constrained in particular taxa, based on both functional and developmental evidence. Phylogenetically, the distribution of carpel orientations does appear to be secondarily constrained among taxa of Lambertia, Banksiinae, Embothrieae/Grevilleae, Cardwellia Gevuina and Bleasdalea (ventral-dorsal), and Helicia, Athertonia and Hollandaea with dorsi-ventral orientations (Fig. 6.6).

Terminal or lateral carpels - In addition to the diverse orientations, two types of carpel initiation occur: terminal and lateral. The carpel in certain taxa is initiated in a lateral position and only part of the floral meristem is converted to carpel. It can be hypothesized that the lateral position of the carpel is a plesiomorphic feature that is retained, if the single-carpellate Proteaceae were derived from a two-carpellate ancestor via loss of one of the carpels. Based on the present phylogenetic evidence, the taxa that initiate the carpel in a lateral position are highly derived, including Embothriinae, Stenocarpus, Buckinghamia, and Grevilleae. Although it could be argued that the lateral carpel has been maintained as a plesiomorphic condition, it would require that the terminal carpel in most of the genera of Grevilleoideae, as well as in Placospermum, Carnarvonia, Sphalmium and Persoonieae/Proteoideae, has been independently derived; that would add at least 10 steps to the phylogeny presented. Alternatively, it can be hypothesized that lateral carpel initiation is a general condition as found in other apocarpous (multicarpellate) angiosperms. Phylogenetically, the lateral initiation of the carpel would then be a reversal to a more general condition and require only one step. Based on principles of parsimony, it is suggested that the lateral carpel is a derived feature in Proteaceae.

Flower-pair origin - The presence of flower pairs among Grevilleoideae is a synapomorphy. Engler (in Engler and Prantl, 1894) identified the flower pairs as a taxonomically important component of Grevilleoideae. The sister group to Grevilleoideae is Sphalmium, and it does not have flower pairs. The inflorescence in Sphalmium is axillary and simple, having 8- to16- flowered racemes. Flowers are single in each bract axil. Sphalmium as the sister group naturally raises interesting questions concerning the origin of the flower pairs among Grevilleoideae. Differing phylogenetic transformation hypotheses explaining the origin of the flower pairs include: 1) reduction of the terminal flower of a dichasium (Haber, 1959, 1961; Eames, 1961); 2) reduced multiple secondary racemoid inflorescence axes to two flowers along a primary axis (Venkata Rao, 1971;

Johnson and Briggs, 1975); and 3) an amplification of first-order meristems. Implied in the first hypothesis (Haber, 1959, 1961) is that all single-flowered taxa (the other four subfamilies) are derived by either a loss of one of the two flowers, or by a separate reduction event from a triad via loss of the two lateral flowers (Haber, 1959, 1961; Eames, 1961). Douglas (Chapters 2-4) demonstrated that there is no developmental evidence supporting morphological reduction events from triads in any of the taxa, and consequently asserts that the inflorescences among Proteaceae show no evidence of cymoid branching. Similar conclusions were made by Johnson and Briggs (1975), Briggs and Johnson (1979), and Venkata Rao (1971). Phylogenetically, there is no support for Haber's hypothesis. The origin of the flower pairs is a derived feature from a single-flowered ancestor.

The second hypothesis (Venkata Rao, 1971; Johnson and Briggs, 1975) asserts that reduction of secondary inflorescence axes along a primary axis is responsible for flower pairs. The production of flower pairs at each node of the primary inflorescence axis is a highly conserved and constrained event in almost all Grevilloideae (exceptions in Lambertia and in some proliferative secondary inflorescence axes in Lomatia fraxinifolia). In addition, there are no intermediate inflorescence forms that support the contention of reduced secondary axes among Proteaceae examined. Douglas (Chapter 2) asserts that for a reduction event to have occurred, the ancestral inflorescence form would probably have been homothetic compound. There are no proteaceous taxa that have homothetic compound inflorescences, and the heterothetic compound inflorescences are distributed sporadically among the basal Proteaceae, the outgroups and among both subfamilies (Fig. 6.6). There is little to no taxonomic evidence that suggests that reduction from secondary axes has taken place, based on the material examined and reviews of inflorescence structure by Venkata Rao (1971) and Johnson and Briggs (1975).

Based on the presumption of secondary axis reduction, Venkata Rao (1971) and Johnson and Briggs (1975) held the opinion that certain Grevilleoideae maintain vestiges in the form of a secondary axis or peduncle (also called the flower-pair stalk), the dorsiventral carpel orientation, and the flower bracts (Fig.6). Based on the present phylogenetic analysis, the flower-pair peduncle appears to have been derived in three separate clades ('ped' in Fig. 6.6). Morphological and anatomical evidence supports this contention. In Musgraveinae, the peduncle is morphologically different from the peduncle in the other taxa. The common-bract is elevated on the peduncle via intercalary growth between the point of common-bract insertion and the principal inflorescence axis (Fig. 6.6). In this respect, the recaulescent common-bract on the peduncle can be interpreted as a unique structure analogous to the flower-bract stalks of some Saururaceae (Tucker, 1975; Liang and Tucker, 1990; Tucker, Douglas and Liang, 1993). The peduncle in the Helicia/Hollandaea/Athertonia clade is not morphologically different from the peduncle in the Cardwellia/Bleasdalea/Gevuina clade. Developmental material of the former clade was not available for comparative examination. Venkata Rao (1971) described anatomical variation between the peduncles of Helicia and Cardwellia. In Helicia, below the base of the peduncle in the primary inflorescence axis, there is a ring of vascular bundles and an increase above this level in the number of bundles by tangential splitting. The large, multi-bundled ring becomes two-lobed and forms two rings, vascular cylinders that each enter a flower. In Cardwellia, the ring of bundles at the base of the peduncle surrounds a strand of sclerenchyma. There is no proliferation of bundle numbers prior to the formation of the two lobes. In addition, as the two floral cylinders depart, several bundles surrounding a sclerenchyma strand persist between the two flowers, and have been interpreted as the residual vasculature of the secondary axis (Venkata Rao, 1971). Based on these comparisons, it can be asserted that the peduncle of the flower-pairs is not homologous, and has been derived two if not three times among Grevilleoideae.

The hypothesis that the flower pairs represent the phylogenetic reduction of secondary branches cannot be dismissed, based on the present studies, but neither do the latter provide much support. Parsimony methods infer that a large increase in number of steps would be necessary to support the postulated reduction pathways of Venkata Rao (1971) and Johnson and Briggs (1975). Six character step increases would be required in the carpel orientation character to maintain the dorsiventral carpel orientation found in Athertonia and Hollandaea as the plesiomorphic condition; eight to twelve step increases would be required if the peduncle represents the plesiomorphic condition, and at least six steps would be required if the presence of non-suppressed floral bracts is the plesiomorphic condition. It should be noted that an increase in taxon sampling could however change the topology of the proposed phylogeny, and this will be investigated in the near future.

A third hypothesis of the origin of the flower pairs proposed by Douglas (chapter 4) asserts that developmentally, the flower-pairs could represent a constrained amplification of a first-order axillary meristem to a two-flowered short-shoot. Similar hypotheses have been proposed for the doubling of flower rows in Zea by Sundberg (1990) and Sundberg, Orr and Lafargue (in press). Amplification of flower numbers from an axillary meristem has also been observed in Musaceae (Barker and Steward, 1962; Ram Mohan, Ram Manasi and Steward, 1962). If such an event occurred in Proteaceae, it can be postulated that there is a physiological delay in the commitment to flowering of an axillary meristem on an inflorescence. The assertion that the flower pairs represent the result of heterochronic amplification and constraint can be examined within a phylogenetic hypothesis. If an amplification event, then the first-order bracts (common bracts) subtending the flower pairs in the basal taxa of Grevilleoideae should have a greater similarity to the floral (first-order) bracts of the single-flowered basal taxa in this analysis, particularly taxa like Sphalmium, Carnarvonia, Placospermum, Persooniinae and

Cennarhenes/Beaupreopsis. Interestingly, there are several features of the first-order bracts that are similar among the taxa (Fig. 6.6). All taxa but Persoonia and Carnarvonia have cuneate-shaped bracts. Secondly, all of the bracts have marginal hairs that develop from the bract tip down. Thirdly, the relatively large *floral bracts* (not the common bracts) in some taxa of Grevilleoideae (namely, Banksieae and the Helicia/Athertonia/Hollandaea clade) appear to be secondarily derived,based on development and parsimony analysis. These larger bracts are generally different than the first order bracts of the same taxa (Douglas pers. obs). The amplification hypothesis will be examined in more detail in future developmental studies.

Summary - Comparative ontogenetic analyses of flowers and inflorescences among Proteaceae provide new evidence of the homology of organization and topological similarities of flowers among taxa. Floral and inflorescence characters that have proven to be difficult to homologize from studies of mature form are easier to interpret, and similarly provide evidence of divergence and convergence, due to similarities or differences in developmental pathways. Ontogenetic evidence and additional mature characters determined from this study, as well as characters used by Johnson and Briggs (1975), are evaluated and coded for phylogenetic analysis. Recent molecular studies have provided evidence for a likely sister group to Proteaceae, so that outgroup analysis and polarization are made possible. The floral, inflorescence, fruit and leaf characters used here provide phylogenetic resolution that is missing in other classifications of Proteaceae. The phylogenetic origin of certain features of Proteaceae are examined using principles of parsimony and developmental likelihood. Developmental evidence of certain morphological trends in the features supports the circumscribed clades. It is concluded that the nectary is a basal or plesiomorphic feature in Proteaceae that has been lost at least three times (Carnarvonia, Sphalmium and the Conospermeae (except Cennarhenes/Beaupreopsis) and that the nectary has been re-derived in

Figure 6.6. Cladogram depicting character changes in inflorescence form, simple or compound; the presence of three types of elongate peduncle of the flower pairs (sketched on right); the presence of marginal hairs (MH - or +) on the first-order pherophyll among all taxa, also called the floral bract in Persoonioideae, Proteoideae, Sphalmioideae and Carnarvonioideae, and called the common bract among Grevilleoideae.

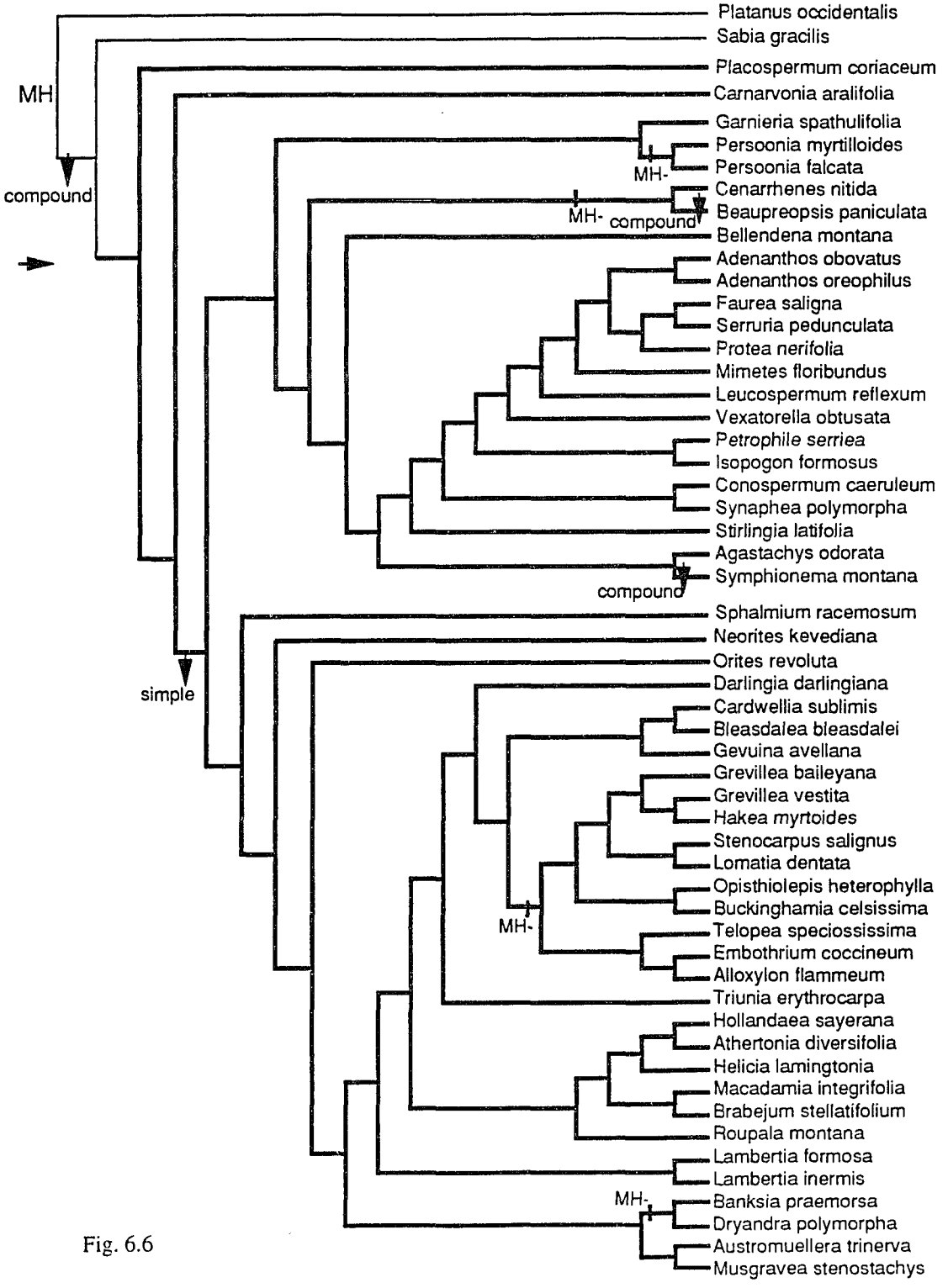
Platanus occidentalis
Sabia gracilis
Placospermum coriaceum
Carnarvonia aralifolia
Garnieria spathulifolia
Persoonia myrtilloides
Persoonia falcata
Cenarrhenes nitida
Beaupreopsis paniculata
Bellendena montana
Adenanthos obovatus
Adenanthos oreophilus
Faurea saligna
Serruria pedunculata
Protea nerifolia
Mimetes floribundus
Leucospermum reflexum
Vexatorella obtusata
Petrophile serriea
Isopogon formosus
Conospermum caeruleum
Synaphea polymorpha
Stirlingia latifolia
Agastachys odorata
Symphionema montana
Sphalmium racemosum
Neorites kevediana
Orites revoluta
Darlingia darlingiana
Cardwellia sublimis
Bleasdalea bleasdalei
Gevuina avellana
Grevillea baileyana
Grevillea vestita
Hakea myrtoides
Stenocarpus salignus
Lomatia dentata
Opisthiolepis heterophylla
Buckinghamia celsissima
Telopea speciossissima
Embothrium coccineum
Alloxylon flammeum
Triunia erythrocarpa
Hollandaea sayerana
Athertonia diversifolia
Helicia lamingtonia
Macadamia integrifolia
Brabejum stellatifolium
Roupala montana
Lambertia formosa
Lambertia inermis
Banksia praemorsa
Dryandra polymorpha
Austromuellera trinerva
Musgravea stenostachys
MH
compound
MH-
MH- compound
compound
simple
MH-

Fig. 6.6

Proteeae/Adenanthiinae. The diverse carpel orientations among Grevilleoideae appear to be taxonomically specific in certain clades and variable at the subfamilial level. The laterally initiated carpels occur in only the most derived taxa of Grevilleoideae, and are therefore inferred to be a derived condition based on changes in developmental timing. The flower pairs of Grevilleoideae are the result of a one-time derived event. There is little extant taxonomic support for the hypothesis of reduced secondary inflorescence branches based on principles of parsimony. A more parsimonious interpretation of the origin of the flower pairs is that they represent a constrained amplification of a first-order meristem. The phylogeny presented includes 49 genera and 53 species of Proteacae. Inclusion of additional taxa will most likely provide additional topological resolution. Similarly, future analyses from molecular studies should elucidate the closest extant taxon to Proteaceae. If Proteaceae is maintained as a basal hamamelid, two nodes from the origin of the angiosperms (Chase et al, 1993), then the unusual flower morphology and topologies most certainly represent one of nature's early flower experiments.

LITERATURE CITED IN CHAPTER 6

Barker, W. G. and F. C. Steward. 1962. Growth and development of the banana plant. 2. The transition from the vegetative to the floral shoot in Musa acuminata cv. Gros Michel. Annals of Botany 26: 413-423.

Bentham, G. and J. D Hooker. 1883. Genera Plantarum. Vol. III. London.

Bessey, C. E. 1915. Phylogenetic taxonomy of flowering plants. Annals of the Missouri Botanical Garden 3: 1-155.

Boothroyd, L. E. 1930. The morphology and anatomy of the inflorescence and flower of the Platanaceae. American Journal of Botany 17: 678-693.

Bremer, K. 1988. The limits of amino acid sequence data in Angiosperm phylogenetic reconstruction. Evolution 42: 795-803.

Bremer, B. 1991. Restriction data from chloroplast DNA for phylogenetic reconstruction: Is there only one accurate way of scoring? Plant Systematics and Evolution 175: 39-54.

Bretzler, E. 1924. Beitrage zur Kenntnis der Gattung Platanus. Botanical Archive 7: 388-417.

Briggs, B. G. and L. A. S. Johnson. 1979. Evolution in the Myrtaceae - Evidence from inflorescence structure. Proceedings of the Linnean Society of New South Wales 102: 157-266.

Briggs, B. G., B. P. M. Hyland and L. A. S. Johnson. 1975. Sphalmium, a distinctive new genus of Proteaceae from North Queensland. Australian Journal of Botany 23: 165-172.

Brown, R. 1810. On the Proteaceae of Jussieu. Transactions of the Linnean Society 10: 15-226.

Bruneau A. and J. J. Doyle. 1993. Cladistic analysis of chloroplast DNA restriction site characters in Erythrina (Leguminosae: Phaseoleae). Systematic Botany 18: 229-247.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y-L Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedren, B. S. Gaut, R. K. Jansen, K-J Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q-Y Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. William, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan, and V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene rbcL. Annals of the Missouri Botanical Garden 80: 526-579.

Chattaway, M. M. 1948. The wood anatomy of the Proteaceae. Australian Journal of Science and Research, Series B, 1: 279-302.

Crisp, M. D. and P. H. Weston. 1987. Waratahs--How many species? Australian National Botanic Gardens (Occ. Publ.) 9: 3-15.

Cronquist, A. 1968. The evolution and classificaton of flowering plants. Boston: Houghton Mifflin.

Cronquist, A. 1988. The evolution and classification of flowering plants. New York: Columbia Universty Press.

Dahlgren, R. 1975. A system of classification of the angiosperms to be used to demostrate the distribution of characters. Bot. Nodiser. 128: 119-146.

Dyer, R. A. 1975. The Genera of Southern African Flowering Plants, vol. 1: 40-47. Government Printer: Pretoria.

Eames, A. J. 1961. Morphology of the Angiosperms. McGraw-Hill Book Company, Inc. New York.

Endress, P. K. and S. Stumpf. 1991. The diversity of stamen structures in 'Lower' Rosidae (Rosales, Fabales, Proteales, Sapindales). Botanical Journal of the Linnean Society 107: 217-293.

Engler, A. , Proteaceae in Engler A. and K. Prantl. 1894. Die Naturlichen Pflanzenfamilien, Vol IV. Leipzig.

Erdtman, G. 1952. Pollen morphology and plant taxonomy, Angiosperms. Stockholm: Almqvist and Wiksell.

Farris, J. S. 1970. Methods for computing Wagner trees. Systematic Zoology 19: 83-92.

Farris, J. S. 1982. Outgroups and parsimony. Systematic Zoology 31: 328-334.

Farris, J. S. 1988. Hennig86. Program and documentation. Port Jefferson Station, New York: Published by the author.

Feuer, S. 1986. Pollen morphology and evolution in the Persoonioideae, Sphalmioideae and Carnarvonioideae (Proteaceae). Pollen et Spores 28: 225-242.

Feuer, S. 1989. Pollen morphology of the Embothrieae (Proteaceae) I. Buckinghamiinae, Stenocarpinae and Lomatiinae. Grana 28: 225-242.

Feuer, S. 1990. Pollen morphology of the Embothrieae (Proteaceae) II. Embothriinae (Embothrium, Oreocallis, Telopea). Grana 29: 19-36.

Foster, A.S. 1952. Foliar venation in angiosperms from an ontogenetic viewpoint. American Journal of Botany 39:752-766.

Gould, S. J. 1977. Ontogeny and phylogeny. Cambridge: Harvard University Press.

Grimes, J. 1992. Metamerism, heterochrony, and inflorescence morphology of the Pithecellobium-complex (Leguminosae: Mimosoideae: Ingeae). Brittonia 44: 140-159.

Haber, J. M. 1959. Comparative anatomy and morphology of flowers and inflorescences of the Proteaceae I. Some Australian taxa. Phytomorphology 9: 325-358.

Haber, J. M. 1961. The comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae II. Some American taxa. Phytomorphology 11: 1-16.

Haber, J. M. 1966 Comparative anatomy and morphology of the flowers and inflorescences of the Proteaceae III. Some African taxa. Phytomorphology 16: 490-527.

Hallier, H. 1912. L'origine et le systeme phyletique des angiospermes. Arch. Neerlandica Sci. Exact. Nat. III. B1: 146-234.

Hennig, W. 1966. Phylogenetic Systematics. University of Illinois Press, Urbana.

Heywood, V. H. 1978. Flowering plants of the world. Mayflower Books, New York.

Hickey, L.J. 1973. Classification of the architecture of dicotyledonous leaves. American Journal of Botany 60: 17-33.

Hickey, L. J. and J.A. Wolfe. 1975. The bases of angiosperm phylogeny: Vegetative morphology. Annals of the Missouri Botanical Gardens 62: 538-589.

Johnson, L. A. S. and B. G. Briggs. 1963. Evolution in the Proteaceae. Australian Journal of Botany 11: 21-61.

Johnson , L. A, S. and B. G. Briggs. 1975. On the Proteaceae--the evolution and classification of a southern family. Botanical Journal of the Linnaean Society 70: 83-182.

Jordaan, P. G. 1944. Die morfologie van die saadknop van South African Proteaceae. D.Sc. Thesis, University of Stellenbosch.

Kaplan, D. R. 1984. The concept of homology and its central role in the elucidation of plant systematic relationships. Pages 51-70 *in* T. Duncan and T. F. Stuessey, eds. Cladistics: Perspectives on the reconstruction of evolutionary history. Columbia University Press, New York.

Kirchoff, B. K. 1983. Allometric growth of the flowers in five genera of the Marantaceae and in Canna (Cannaceae). Botanical Gazette 144:110-118.

Lanyon, J. W. 1979. The wood anatomy of three proteaceous timbers, Placospermum coriaceum, Dilobeia thouarsii and Garnieria spathulaefolia. IAWA Bulletin 2: 27-33.

Lawrence, G. H. M. 1954. Taxonomy of the Flowering Plants. New York: Columbia University Press.

Liang, H.-X. and S. C. Tucker. 1990. Comparative study of the floral vasculature in Saururaceae. American Journal of Botany 77: 607-623.

Maddison, W. P., M. J. Donoghue and D. R. Maddison. 1984. Outgroup analysis and parsimony. Systematic Zoology 33: 83-103.

Maddison, W. P. and D. R. Maddison. 1992. MacClade, Version 3. Program and documentation. Sinauer Associates, Sunderland.

Manning, J. C. and G. J. Brits. 1993. Seed coat development in Leucospermum cordifolium (Knight) Fourcade (Proteaceae) and a clarification of the seed covering structures in Proteaceae. Botanical Journal of the Linnean Society 112: 139-148.

Meacham, C. A. 1984. The role of hypothesized direction of characters in the estimation of evolutionary history. Taxon 33: 26-38.

Melville,. 1975. The distribution of Australian relict plants and its bearing on angioseprm evolution. Botancial Journal of the Linnean Society 71: 67-88.

Mennega, A. M. W. 1966. Wood anatomy of the genus Euplassa and its relation to other Proteaceae of the Guianas and Brazil. Acta Botanica Neerlandica 15: 117-129.

Midgeley, J. J. 1987. The deviation, utility and implications of a divergence index for the fynbos genus Leucadendron (Proteaceae). Botanical Journal of the Linnean Society 95: 137-152.

Nelson, E. C. 1978. A Taxonomic revision of the genus Adenanthos (Proteaceae). Brunonia 1: 303-406.

Nixon, K. C. 1992. Clados version 1.2. Program and documentation. Ithaca, New York: Published by the author.

Nixon, K. C. and J. M. Carpenter. 1993. On outgroups. Cladistics 9: 413-426.

Nixon, K. C. and J. I. Davis. 1991. Polymorphic taxa, missing valuses and cladistic analysis. Cladistics 7: 233-241.

Nixon, K. C. and Q. D. Wheeler. 1992. Extinction and the origin of species. Pages 119-143 *in* M. J. Novacek and Q. D. Wheeler, eds. Extinction and phylogeny. Columbia University Press, New York.

Patterson, C. 1982. Morphologcial characters and homology. Pages 221-274 *in* K. A. Joysey and A. E. Friday eds. Problems of phylogenetic reconstruction. Academic Press, London.

Patterson, C. 1987. Introduction. *In*, C. Patterson, ed. Molecules and morphology in evolution: Conflict or compromise? Cambridge University Press, Cambridge.

Platnick, N. I., C. I. Griswold, and J. A. Coddington. 1991. On missing entries in cladistic analysis. Cladistics 7: 337-343.

Pulle, A. A. 1950. Compendium van de Terminologie. Nomenclateur en Systematick der Zadplanten. Utrecht.

Ram Mohan, H. Y., Ram Manasi and F. C. Steward. 1962. Growth and development of the banana plant. 3A. The origin of the inflorescence and the development of the flowers. Annals of Botany 26: 657-673.

Rendle, A. B. 1959. Classification of flowering plants. Vol. II Dicotyledons. Cambridge.

Rourke, J. P. 1972. Taxonomic studies on Leucospermum R. Br. Journal of South African Botany, Supplement, 8: 1-194.

Rourke, J. P. 1984a. A revision of the genus Mimetes Salis. (Proteaceae). Journal of South African Botany 50: 171-236.

Rourke, J. P. 1984b. Vexatorella Rourke, a new genus of the Proteaceae. Journal of South African Botany 50: 373-391.

Schönland, S. 1883. Ueber die Entwicklug der Blüten und Frucht bei den Platanaceae. Botanische Jahrbücher der Systematik 4: 308-327.

Schwarzwalder, R. N. Jr. and Dilcher, D. L. 1991. Systematic placement of the Platanaceae in the Hamamelidae. Annals of the Missouri Botanic Garden 78: 962-969.

Stebbins, G. L. 1951. Natural selection and the differentiation of Angiosperm families. Evolution 5: 299-324.

Stevens, P. F. 1991. Character states, morphological variation, and phylogenetic analysis: A review. Systematic Botany 16: 553-583.

Sundberg, M. D. 1990. Inflorescence development in Zea diploperennis in relation to other Zea species. Maydica 35: 99-111.

Sundberg, M. D., C. La Fargue and A. R. Orr. in press. Inflorescence development in the "standard exotic" maize, Argentine popcorn (Poaceae). American Journal of Botany.

Swofford, D. L. 1991. PAUP: Phylogenetic analysis using parsimony, version 3.0. Illinois Natural History Survey, Champaign, Illinois.

Thorne, R. 1968. Synopsis of a putatively phylogenetic classification of the flowering plants. Aliso 6: 57-66.

Tucker, S. C. 1975. Floral development in Saururus cernuus. I. Floral initiation and stamen development. American Journal of Botany 62: 993-1007.

Tucker, S. C. 1988. Loss versus suppression of floral organs. In, P. Leins, S. C. Tucker and P. K. Endress, (eds.), Aspects of Floral Development, Berlin: Gebrüder Borntraeger.

Tucker, S. C. and A. W. Douglas. In Press. Floral structure, development, and relationships of paleohers: Saruma, Cabomba, Lactoris and selected Piperales. *In* L. Hickey and D. W. Taylor, eds. Flowering plant origin, phylogeny, and evolution.

Tucker, S. C., A. W. Douglas and Liang Han-Xing. 1993. Utility of ontogenetic and conventional characters in determining phylogenetic relationships of Saururaceae and Piperaceae (Piperales). Systematic Botany 18: 614-641.

Venkata Rao, C. 1957. Cytotaxonomy of the Proteaceae. Proceedings of the Linnaean Society N.S.W. 82: 257-271.

Venkata Rao, C. 1960. Studies in the Proteaceae I. Tribe Persoonieae. Proceedings of the National Institute of Sciences of India 26B: 300-337.

Venkata Rao, C. 1961. Studies in the Proteaceae II. Tribes Placospermeae and Conospermeae. Proceedings of the National Institute of Sciences of India 27B: 126-151.

Venkata Rao, C. 1967. Morphology of the nectary in Proteaceae. New Phytologist 66: 99-108.

Venkata Rao, C. 1971. Proteaceae. Botanical Monograph #6. Council of Scientific and Industrial Research. New Delhi.

Weberling, F. 1989. Morphology of Flowers and Inflorescences. Engl. Trans. by Pankhurst. Great Britain: Cambridge University Press.

Weston, P. W. 1983. The systematics and biogeography of the Persooniinae (Proteaceae). Dissertation. University of Sydney, N.S.W., Australia.

Weston, P. W. and L. A. S. Johnson. 1991. Taxonomic changes in Persoonia (Proeaceae) in New South Wales. Telopea 4: 269-306.

Wettstein, R. 1935. Handbuch der systematischen Botanik. Leipzig.

APPENDIX 6.1

Data matrix - Character state distribution of 155 characters among the 55 taxa examined.

```
Platanus occidentalis
00000 00?00 ?0??? 02??0 ???00 00??? 0?000 00000 00000 10000
01000 00??? 00000 03101 00000 00000 10000 00001 0210? ?0?05
?2011 0200? 00120 11210 11100 22104 12310 40201 10002 21111
00?00

Sabia racemosa
13000 00000 00??0 01??0 00000 00000 00000 00011 01110 00001
00000 01?0? 00000 00000 00011 00100 10000 00000 00111 10?12
?0101 12000 10021 02202 10020 03202 22330 00100 00000 20100
0?000

Placospermum coriaceum
10001 11200 02003 20000 01100 01001 01010 00100 06010 10010
04000 02101 01000 00000 00002 00410 00001 ?0000 00011 11?02
?1001 00001 00020 21113 30001 01113 13330 00110 00000 21000
10111

Garnieria spathulifolia
10001 111?? 00001 02200 02104 00001 01010 00110 12000 10011
02000 02110 00000 00000 10021 10110 00100 10000 01111 01?02
?1101 12001 1???? ????? ????? ????? ????? ????? ??00? ??201
00111

Persoonia myrtilloides
11001 11210 00101 02120 02104 00001 01010 00110 12000 10011
03000 02110 00000 00000 20021 10100 00100 10000 0010? 00?00
?0??1 12001 10102 02201 10002 01101 11043 01010 00000 20100
00111

Persoonia falcata
10001 11210 2?103 02220 01104 00101 01010 00110 05000 10011
04000 02110 01000 00000 10021 11100 00100 10000 0010? 00?00
?0??1 12001 ??102 02202 20010 00000 21343 00010 01010 20100
00?11

Bellendena montana
11001 11000 00000 20000 00001 00?3? 01000 00000 11000 10010
0?000 02110 00000 00000 10011 11100 10000 00001 00110 00?05
?1011 22000 10102 02103 21000 00000 01040 00001 11012 31001
00111

Adenanthos obovatus
12001 11211 00122 12000 10000 03001 01110 11100 01000 10110
03101 02110 10080 22101 00132 03131 00111 01000 02111 00?03
?2011 32101 10102 02100 20010 20000 ?1043 01010 01011 31001
02?11

Adenanthos oreophilus
11001 11211 00122 12200 10000 03001 01110 11100 01000 10110
03101 02110 10020 13101 00132 01331 00111 01001 02111 00?03
```

?2010 32101 1???? ????? ????? ????? ????? ????? ??02? ??001
02?11

Cenarrhenes nitida
11001 11011 00001 20000 00101 00101 01000 00010 04110 00111
13010 0211? 00000 00000 00011 01100 10101 00001 11100 01?03
?1101 12001 1???? ????? ????? ????? ????? ????0 ??00? ??001
00?11

Beaupreopsis paniculata
11101 11011 10001 00000 00101 00101 01000 00010 04110 00111
13010 0211? 00000 03101 00011 01100 00101 ?0001 00100 01?03
?1101 22?01 1???? ????? ????? ????? ????? ????0 ??00? ??101
01?11

Agastachys odorata
11?01 11000 00001 200?0 00000 00?3? 01010 00010 12000 00111
12000 02110 00000 00000 00053 01130 10000 00001 02010 01?02
?1110 32001 1?102 02103 20001 03010 01040 00000 01000 31000
01?11

Symphionema montana
11?01 11000 00001 20020 00000 00?3? 01000 00010 12000 00111
22000 02110 01000 00000 00061 01100 10001 00001 12000 21?02
?1110 32001 1?102 02100 21000 00010 01040 30002 11012 31001
00?11

Stirlingia latifolia
12?01 11210 10102 20000 10101 00?3? 01010 00010 22000 00111
22000 02110 00000 03101 00051 01130 20200 00001 02000 20?02
?2011 32101 11100 02103 20000 00015 00043 01012 12010 31001
00?11

Petrophile serriea
11001 11201 00001 12201 ?2?0? 00?3? 01?10 11000 01001 00010
02100 02110 ?0161 13110 00061 01130 10001 ??001 0200? 0??0?
?3011 32101 1???? ????? ????? ????? ????? ????? ??0?? ??000
00?11

Isopogon formosus
11001 11201 00002 20001 1010? 00?3? 01010 11000 01001 00110
02100 02110 00161 03110 00161 01130 10001 0?001 02010 00?03
?3011 32101 10102 02101 20000 23010 00043 01010 02010 31200
00?11

Conospermum caeruleum
12?01 11220 12202 11000 00101 01?3? 01010 00000 22000 10111
22000 12110 01000 01110 00064 01130 10001 00001 02010 10?03
?2011 32101 11102 02103 20001 00010 20040 00010 01010 51001
03?11

Synaphea polymorpha
11?01 11220 12202 12100 00101 00?3? 01010 00010 22010 10111
22000 12110 00000 01110 00053 01130 10001 00001 02010 10?02
?2101 32?01 11?00 11200 21000 00015 00330 00012 11012 31001
00?11

Faurea saligna
10001 11201 00121 02200 10000 03000 01010 11110 01000 1011?
0?001 0211? 00020 13111 00032 02201 10111 ?1001 0111? 00?03
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0?111

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?1111

Mimetes floribundus
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Serruria pedunculata
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Sphalmium racemosum
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01000 0211? 00000 03101 00032 00001 00201 ?0000 00111 01?03
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00111

Orites revoluta
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Neorites kevediana
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Darlingia darlingiana
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01011 00001 00120 11001 00000 02215 22110 00100 00000 2?001
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Cardwellia sublimis
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Grevillea baileyana
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01101 00?01 00102 0021? 20010 01113 12230 00110 00000 21000
10111

Grevillea vestita
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Hakea myrtoides
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10111

Banksia praemorsa
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Dryandra polymorpha
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Austromuellera trinerva
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Musgravea stenostachys
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Opisthiolepis heterophylla
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Buckinghamia celsissima
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Telopea speciossissima
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00011

Embothrium coccineum
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01010 00001 00120 11111 20001 23105 12230 00100 00000 21021
04111

Alloxylon flammeum
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01111

Stenocarpus salignus
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00?11

Hollandaea sayerana
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Triunia erythrocarpa
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Bleasdalea bleasdalei
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Gevuina avellana
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Macadamia integrifolia
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02111

Brabejum stellatifolium
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03010 12?10 00020 23101 00042 02001 10001 00000 00111 02005
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Roupala montana
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??111

Lambertia formosa
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Lambertia inermis
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00?11

Lomatia dentata
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00011

Helicia lamingtonia
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00011

Athertonia diversifolia
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11101 01001 10100 00204 22100 21204 ?2110 40100 00000 10???
??011

APPENDIX 6.2

CHARACTERS USED: The characters used in the phylogenetic analysis are listed. The characters are grouped according to organs. Sources for the data came primarily from my independent observations although other characters like wood, pollen, fruit and anatomical characters were obtained from the following abbreviated sources (Johnson and Briggs, 1963, 1975-**JB**; Venkata Rao-**VR**; Haber-**JH**; Chattaway-**MC**; Sylvia Feuer-**SF**). ** represents characters excluded due a high proportion of missing states. Characters in italics represent developmental characters.

1 **Stipules** 0-present; 1-absent

2 **Habit** 0-tree; 1-shrub; 2-lignoshrub; 3-liana

3 **Phyllotaxis** 0-spiral; 1-whorled; 2-opposite

4 **Flower pairs** 0-absent; 1-present

Tepal characters

5 **Number of perianth whorls** 0-two; 1-one

6 **Number of perianth parts/whorl** 0-five; 1-four

7 **Aestivation** 0-imbricate; 1-valvate

8 **Reflexion of tepals at point of insertion on receptacle** 0-present; 1-half way or partial; 2-no reflexion at base

9 **Upper tepal (not limb) bending** 0-none; 1-all four bend; 2-lower three bend; 3-ad lobe bends

10 **Curvature of tepals at anthesis** 0-absent; 1-smooth curve or circinate

11 **Tepal limb lengths** 0-all ~equal; 1-adaxial longest; 2-abaxial longest; 3-laterals longest and shortest (closest to principal axis in Grevilleoideae)

12 **Total tepal length** 0-all equal; 1-adaxial longest, other three equal; 2-adaxial longest, others shorter; 3-laterals longest

13 **Basal tepal width** 0-uniformly broadened at base in all tepals; 1-broadened in adaxial tepal only

14 **Tepal limb** 0-equal width to claw; 1-claw broader; 2-claw broader than limb in all but abaxial tepal

15 **Perianth tube (Perigon)** 0-no tube; 1-short straight tube; 2-long straight tube; 3-curved tube

16 **Trichomes or hairs on outer epidermis of tepals** 0-present, short; 1-present, elongate; 2-absent

17 ***Hairs persistence on tepals*** 0-glabrous; 1-ephemeral; 2-persistent

18 ***Hair density on tepals*** 0-does not occur; 1-diffuse because of early cessation; 2-dense because hair production continues

19 ***Trichomes on inner epidermis of tepals*** 0-absent; 1-tapered hairs; 2-papillate; 3-arcuate hairs; 4-globose(narrow stalk and swollen tip)

20 ***Zonal growth between tepals*** 0-absent; 1-elongate tube forms

21 **Limb-claw distinction** (VR; H) 0-no differentiation; 1-limb slightly different from claw; 2-extreme differentiation between limb and claw

22 **Vorläuferspitze** 0-absent; 1-present; 2-modified extension

23 **Distal tepal tip** 0-not incurved; 1-tepal tips incurved

24 **Floral receptacle symmetry** 0-actinomorphic; 1-zygomorphic, expanded proximally to the ventral side of the carpel; 2- zygomorphic, expands proximal to the dorsal side of the carpel

25 **Differentiation of tepal tip** 0-glabrous; 1-extended cells; 2-hairs; 3-extended cells and hairs; 4-tip extension and extended cells

26 **Anthesal reflexion of tepals** 0-lobes reflex away; 1-ad. lobes of pair stay straight

27 **Interlocked margins at anthesis** 0-freely unlock; 1-all stay locked; 2-lats. and ad. locked, ab. free; 3- margin between ab. and lat. free, lats. and ad. locked,; 4-ad free margin between ad. and lat. free ad, ab, lat. locked one lat. free all locked, lat. margin free

Nectary characters

28 **Gland width** 0-laminar; 1-terete

29 **Number of nectary lobes** 0-4; 1-3; 2-2; 3-1; 4-none

30 **Gland lobe morphology** 0-tapered; 1-cuneate; 2-elongate

31 **Gland epicarpelous** 0-free; 1-epicarpellous

32 **Intercalary growth between glands** 0-present; 1-absent; 2-partial

Stamen characters

33 **Filament attachment** 0-glabrous; 1-hairs at interface

34 **Intercalary adnation of filament to tepal** 0-free; 1-partially adnate and partially free; 2-adnate entirely

35 ***Nectary initiation*** 0-simultaneous; 1-sequential

36 **Filament tepal adnation form** 0-recognizable form; 1-both recognizable at top of tepal and not recognizable at the base; 2-not recognizable

37 ***Filament tepal attachment*** 0-not lobed; 1-lobed

38 **Filament shape in cross section** (VR, H, ps) 0-isoradial; 1-isobilateral

39 **Longitudinal shape of the filament** 0-uniform or parallel; 1-taaapered and distally becomes narrower at point of attachment to anther

40 **Longitudinal shape of the filament 2** 0-equal or parallel; 1-broadens towards point of attachment with anther

41 **Position of top of connective relative to microsporangia** 0-above microsporangia; 1-even with the tops of microsporangia; 2-below tops of microsporangia

42 **Shape of connective appendage** 0-cap-shaped; 1-rounded or no modification; 2-flattened or lacking; 2-pyramidal; 3-tapered or acute; 4-laminar and elongate; 5-elongate and obtuse

43 **Connective shape** taken from the base of the microsporangia to the top of the microsporangia 0-parallel and straight; 1-distally acute; 2-basally acute; 3-broad in middle, basally and distally acute.

44 **Microsporangia positions** 0-parallel; 1-converge distally; 2-converge distally and basally

45 **Tepal and back of connective fused (post-genital)** 0-not fused 1-fused

46 **Filament bends at top** 0-bends centrifugally; 1-straight

47 **Abaxial connective form variation** 0-uniform 1-connective ridges

48 **Filament connation** 0-free; 1-connate at top

49 **Dehiscence pattern** 0-longitudinal valvate; 1-longitudinal lines

50 **Over the top dehiscence** (almost saddle-shaped anthers sensu Kunze, 1978) 0-absent; 1-in front of connective; 2-splits connective or sterile tissue

51 **Anther connation** 0-free; 1-fused at base and top; 2-fused along length of anther; 3-fused and common chambers between neighboring anthers.

52 **Anther sac position** 0-extrorse; 1-tangentially latrorse; 2-circumferentially latrorse; 3-right-angle introrse; 4-radially or acute introrse

53 **Anther epidermis** 0-regular or smooth; 1-verrucate

54 **Microsporangia size equality** 0-abaxial pair equal to adaxial pair; 1-abaxial microsporangia shorter than adaxial microsporangia

Carpel characters

55 ***Pre-anthetic style curvature*** 0-straight; 1-adaxially curving

56 ***Pre-anthetic style curvature*** 0-straight; 1-abaxially curving

57 **Carpel number** 0-several; 1-two; 2-one

58 ***Carpel cleft position*** 0-abaxial position; 1-adaxial position; 2-lateral position; 3-oblique position

59 ***Carpel initiation*** 0-lateral; 1-terminal

60 ***Early form of carpel*** 0-peltate-cross zone; 1-epeltate, cleft extends to carpel base

61 **Adaxial style curvature** 0-straight; 1-adaxial

62 **Abaxial style curvature** 0-straight; 1-curves abaxially

63 **Basal ridge hairs on style (pollen presenter)** 0-absent; 1-present

64 **Pollen-presenter type** 0-absent; 1-elongate club; 2-orbicular club; 3-spatula; 4- disc shaped or phalliform; 5-elongate brush or ridged; 6-elongate flattened area

65 ***Trichomes on top of style associated with pollen-presenter*** 0-absent; 1-multiseriate hairs; 2- papillate

66 **Style exsertion/preanthesis** 0-not exserted; 1- slight exsertion; 2- highly exserted

67 **Locations of ovary hairs** 0-glabrous; 1-hairy at top; 2-hairy at bottom; 3-hairy all over; 4-ephemeral

68 **Long ovary hairs** 0-glabrous; 1-long, narrow hairs

69 **Short ovary hairs** 0-glabrous; 1-short, stubby hairs

70 **Receptacular hairs** 0-glabrous; 1-long, narrow; 2-short, narrow; 3-thick, stubby

71 **Stipe** 0-sessile; 1- short stipe; 2-long stipe

72 **Stipe hairs** 0-glabrous; 1-hairy

73 **Style suture** 0-furrowed the whole way, not completely sealed; 1-completely sealed or not furrowed all the way

74 **Stigma type** 0-decurrent; 1-punctiform; 2-capitate; 3-long bifid slit; 4-short bifid slit; 5-discoid and/or hooded; 6-poricidal

75 **Stigma shape** 0-platanoid decurrent; 1- round; 2-slit; 3-ovoid; 4-hooded

76 ***Substipitate carpel cushion*** 0-absent; 1-present

77 **Pollen relaease relative to stigma** 0-completely separate; 1-stigmatic area separated; 2- stigmatic area close to pollen; 3-pollen covers stigma

78 ***Stigma position on carpel*** 0-adaxial (ventral) and distal; 1-ventral, not extending to summit; 2-ventral and extending on summit of carpel; 3-on dorsal and ventral side, over the summit of the carpel; 4-recurved to abaxial or dorsal side of carpel

79 **Ovule number** 0-two; 1-many; 2-four; 3-one

80 **Stigmatic area expansion** 0-open stigma at anthesis; 1-stigma opens after anthesis

81 **Ovule position** 0-lateral; 1-high lateral or apical; 2-low lateral or basal

82 ***Ovule displacement*** 0-not displaced, stay in rows; 1-displaced tangentially

83 **Ovule type** 0-orthotropous; 1-hemitropous; 2-anatropous

84 **Broad diametric hairs around top of ovary** 0-absent; 1-present

85 **Ovule funiculus length** 0-long; 1-short

86 **Outer/inner integument arrangement** 0-inner integument longer and solely forms micropyle; 1-outer integument is longer than inner integumente and surrounds mouth of micropyle

87 **Preanthetic lobes on style basal to stigma** 0-absent; 1-present

88 **Papillae around stigma** 0-absent; 1-present

89 **Hairs around stigmatic area** 0-absent; 1-present

Inflorescence and bract characters Inflorescence terminology used here is defined by Briggs and Johnson (1979), Weston (1983, 1989), Weberling (1989) and Grimes (1992). For a review of the flower pair characters of Grevilleoideae, reference is made to Douglas (Chapter 4).

90 **Inflorescence relative growth unit** 0-anauxotelic; 1-auxotelic

91 **Bract form at anthesis** 0-stays erect; 1-bends at anthesis

92 **Pedicel** 0-present; 1-short or modified; 2-absent

93 **Pedicel hairs** 0-absent; 1-present

94 ****Hairs on first-order bract margins** 0-absent; 1-present

95 **Trichomes on tip of bract** 0-glabrous; 1-hairy

96 **Subunitary buds on inflorescence or secondary branching** 0-no secondary inflorescence branching, simple inflorescences; 1-secondary inflorescence branches present (heterothetic double raceme); 2-tertiary to quaternary branching (heterothetic triple or quadruple raceme)

97 **Anthotaxy and internode spacing on inflorescence** 0-equal spacing, spiral; 1-distichous or tetrastichous; 2-whorled

98 ****Common bract elevated on peduncle (common bract stalk,** *see text*) 0-sessile on primary axis; 1-common-bract elevated

99 **Flower-bract stalk (concaulescence)** 0-absent; 1-present

100 **Form of floral bracts** 0-leaf; 1-reduced or small leaf; 2-acute; 3-obtuse and attenuate; 4-highly acute; 5-suppressed after initiation

101 ****Pairs fused (peduncle)** 0-absent; 1-present; 2-short or modified 3-lacking

102 **Inflorescence typology** 0-uncondensed, internodes same between vegetative region and flowering region; 1-condensed raceme, short internodes; 2-aggregate head; 3-capitulum; 4-spadix

103 **Terminal inflorescence** (not to be confused with terminal flower) 0-present; 1- absent

104 **Axillary inflorescences** 0-present; 1-absent

105 **Frondobracteose inflorescence** 0-present; 1-absent

Fruit characters

106 **Fruit type** 0-follicle; 1-succulent, drupe; 2-nonsucculent pseudo-follicle; 3-achene

107 **Dehiscence of fruit** 0-dehiscent; 1-tardily dehiscent; 2-indehiscent

108 **Fruit hairy coma and or awns** 0-lacking coma or awns; 1-coma present; 2 awns present.

109 **Cork on exocarp** 0-lacking cork; 1-cork present

110 **Endosperm** (VR) 0-lacking; 1-present

111 **Seed dispersal** 0-winged; 1-not winged

Leaf architecture characters Leaf vascular architecture in Proteaceae was investigated and characters were delimited according to Foster (1952), Hickey (1973) and Hickey and Wolfe (1975). Leaf characters were obtained by external observation of uncleared leaves. Finer leaf details such as higher order venation patterns and areolation were examined in cleared leaves.

112 **Secondary veins dichotomous** 0-absent; 1-present

113 **Intersecondary origin** 0-from primary vein; 1-origin from the inner part of secondary arches or obmedial intersecondary; 2-from both 0 and 1

114 **Tertiary vein origin** 0-from secondaries; 1-from intersecondaries; 2- from both the intersecondaries and secondaries

115 **Side of secondary vein the tertiary veins join** 0-both sides of secondary vein; 1-primarily (80%) join on the abmedial side; 2-primarily join on the admedial side

116 **Tertiary admedial angle** 0-acute; 1-right angle; 2-obtuse angle

117 **Tertiary abmedial angle** 0-acute; 1-right angle; 2-obtuse angle; 3-variable

118 **Leaf apex** 0-lacking tooth-apex; 1-mucronate and rounded; 2-attenuate

119 **Leaf lamina base symmetry** 0-symmetrical lamina; 1-lamina originates at different heights on petiole; 2-lamina at different heights and asymmetrical in width

120 **Leaf apex excluding tip** 0-acute; 1-obtuse; 2-attenuate; 3-rounded; 4-acuminate; 5-mucronate; 6-retuse

121 **Leaf base shape** 0-acute; 1-obtuse-rounded; 2-cuneate; 3-acute and/or decurrent

122 **Leaf margin** 0-entire; 1-toothed

123 **Shape of teeth** 0-none; 1-serrate

124 **Secondary veins angle of divergence (middle 1/2 of leaf examined)** 0-acute, less than 80 degrees; 1-narrow, less than 45 degrees; 2-greater than 81 degrees

125 **Variation in angle of divergence of secondary veins** 0-no variation; 1-uppers more obtuse; 2-uppers more acute; 3-lowest pair more acute only

126 **Course of secondaries** 0-straight; 1-recurved; 2-uniform curved

127 **Intersection angles of brochidodromous secondary loops** 0-no loops formed; 1-acute angle; 2-right angle; 3-obtuse angle

128 **Intersecondary veins** 0-absent; 1-simple presence; 2-composite presence

129 **Intramarginal vein** 0-absent; 1-occasionally present or discontinuous; 1-always present

130 **Third order branching pattern** 0-ramified transverse; 1-ramified exmedial; 2-ramified admedial; 3-reticulate random; 4-percurrent or forked; 5-orthogonal reticulate; 6-composite intersecondary; 7-transverse

131 **Ultimate venation of margins** 0-incomplete; 1-looped; 2-fimbriate; 3-fimbriate but a component of the secondary and tertiary arches

132 **Veinlets or vein endings** 0-lacking; 1-simple linear or curved; 2-branched once; 3-branched twice; 4-branched thrice

133 **Areoles** 0-lacking; 1-well developed; 2-imperfect; 3-incomplete or not closed

134 **Areole shape** 0-triangular; 1-quadrangular; 2-pentagonal; 3-irregular; 4-lacking

135 **Petiole** 0-normal or regular; 1-inflated; 2-winged; 3-lacking an elongate petiole, lamina sessile on stem

136 **Serrations** 0-lacking; 1-concave on the apical side, concave on the basal side; 1-acuminate on the apical side, concave on the basal side; 2-convex on the apical side, concave on the basal side; 3-convex on the basal side, concave on the apical side; 4-regular indentations

137 **Leaf apical callus** 0-absent; 1-present

138 **Secondary arches** 0-absent; 1-present over first brochidodromous arch

139 **Margin thickness** 0-no difference between lamina and margin; 1-margin thickened

140 **Leaf margin** 0-entire; 1-lobed less than a third to midvein; 2-lobed between two-thirds and one-third to midvein; 3-lobes extend to midvein

141 **Lobe position on leaf** 0-none; 1-whole leaf; 2-top half of leaf only

142 **Number of veins entering leaf** (VR) 0-one; 1-one major vein, two minor veins; 2-one major vein, several minor veins; 3-numerous veins, no distinct primary vein

143 **Adult leaf shape** 0-simple; 1-compound

144 **Leaf vein facial** 0-dorsiventral; 1-bifacial; 2-centric

145 **Primary venation branching pattern** 0-one vein, no split; 1-suprabasal split of primary; 2-multiple suprabasal splits of primary vein; 3-palanictodromous

146 **Secondary vein branching pattern** 0-craspedodromous; 1-semicraspedodromous; 2-brochidodromous; 3-eucamptodromous; 4-cladodromous; 5-camptodromous mixed with brochidromous

147 **Secondary vein course** 0-straight or curved; 1-sinuous

Pollen characters

148 ****Pollen type** 0-porate; 1-colpoidate lolongate; 2-colpoidate lalongate

149 ****Pollen arms** 0-three arms (triamburate); 1-spherical; 2-two arms (biamburate)

150 ****Pollen polarity** 0-isopolar; 1-subisopolar

151 ****Pollen pore membranes** (endexine) 0-not protuberant; 1-protuberant

152 ****Pollen shape** 0-oblate; 1-suboblate; 2-peroblate; 3-spheroidal; 4-elongate

153 **Perforation plates on vessels** 0-scalariform; 1-simple

154 **Number of stamens** 0-more than four; 1-four

CHAPTER 7

SUMMARY

Comparative floral and inflorescence ontogeny of flowers was studied in 78 species in 62 genera representing all tribes and 25 of 27 subtribes of Proteaceae. Distinct morphological features examined include the patterns of floral initiation, the developmental bases of the perianth and nectaries, the developmental events responsible for specialized inflorescence features, and the developmental events influencing carpel orientations. The results of the comparative ontogenetic and morphological studies provide a clarified interpretation of the developmental and phylogenetic diversity of the unique flowers of Proteaceae.

Developmental comparisons provide evidence that the flowers are four-merous, the tepals being initiated in a sequence of two dimerous whorls. The tepals in the median frontal sagittal plane are initiated first, followed by the initiation of the adaxial and abaxial tepals. The four stamens are initiated in a similar sequence as the tepals, and the stamens are in the same orthostichy; thus the stamens are superposed. Each flower has a single carpel. From both early comparative ontogenies and phylogenetic analyses, the flowers of Proteaceae appear to be primitively simple and apetalous.

The orientation of the flowers among Grevilleoideae is clarified via comparative ontogenetic studies. A synapomorphic character of the subfamily is the two-flowered short-shoot in the axil of a first-order bract along a primary axis (flower pairs). Developmental comparisons of the earliest stages of inflorescence and floral organogenesis reveal that the flower pairs are two-flowered short-shoots and that the flowers are subtended by reduced floral bracts. Thus, the flowers are dorsiventrally aligned like flowers in the other proteaceous subfamilies. The origin of the flower pairs was a one-time event; it could either be be the product of a reduction of secondary inflorescence axes or the product of an amplification, via a constrained proliferation of first-order axillary meristems to two-flowered short-shoots .

Stamen morphogenesis is diverse among taxa, including adnation of the filament to the tepal, connation of neigboring filaments, connation of neighboring anthers, and partial sterility of single anthers via suppression of thecal development on one side, or lateral reduction. Many of the diverse features involved in stamen morphogenesis are associated with primary and secondary pollen presentation.

There is only a single carpel in all proteaceous taxa. There are developmentally diverse events involved in initiation and subsequent morphogenesis of the carpels among different taxa. In most taxa, the carpel is the product of conversion of the terminal floral apex after stamen initiation. In the other taxa, the carpel is initiated in a lateral position on the floral apex. The carpel is oriented dorsiventrally, the cleft facing the adaxial tepal among flowers of taxa in Proteoideae, Persoonioideae, Carnarvonioideae, Sphalmioideae, and the genera Hollandaea and Athertonia of Grevilleoideae. Five additional cleft orientations occur among various taxa of Grevilleoideae. The orientation of the carpel (position of the cleft) appears to be correlated with the broadest diameter of the floral meristem after stamen initiation, the cleft developing in the plane perpendicular to the longest diameter. It is hypothesized that the organization of the carpel within flowers of Grevilleoideae is loosely regulated in general, although certain orientations have become fixed or constrained in some lineages, probably related to functional traits including the expression of zygomorphy associated with pollination syndromes.

The carpels are diverse in the development of secondary pollen presentation modifications (pollen-presenters) on the distal end of the style. Phylogenetically, secondary pollen presentation on the carpel has been derived three times: once in Petrophilinae/Proteeae/Adenanthiinae; once in Carnarvonioideae; and once in Grevilleoideae. Taxa lacking a secondary pollen presenter have diverse forms of stigmas including porate, punctiform, abaxially bifid, capitate, disk-shaped, hooded and

spatulate. The primary pollen presentation depends upon the position of the stamens around the carpel, including connation of the upper portions of each filament and the lateral sides of the anthers.

In most taxa, nectaries are initiated relatively late in development after enlargement of the floral receptacle and/or after zonal growth proximal to stamen attachment on the tepal. Thus, the nectaries are not reduced petals or stamens. From phylogenetic comparisons, the nectary was present in the common-ancestor of Proteaceae and has been subsequently lost in Sphalmium, Carnarvonia and Conospermeae/Bellendena. In addition, the nectary appears to have been secondarily derived among Proteeae and Adenanthiinae, based on the developmental processes of initiation from a short hypanthium, the more laminar morphology, a lack of vasculature, and phylogenetic evidence. In the basal taxa, the nectary is initiated from a flat floral receptacle

A phylogenetic analysis of the family is presented. A total of 154 morphological characters among 55 taxa provided 22 most-parsimonious hypotheses of relationships. Characters were based on the ontogenetic analyses at two levels. At the first level, the orientations of the flowers and the carpels among Grevilleoideae were determined based on ontogenetic comparisons (Chapters 4-5). These analyses provided the rationale for fundamental homology of organization, so that homologous floral characters involved with the orientations of flowers and symmetries could be compared. At the second ontogenetic level, morphological characters were established based on determining the developmental events that produce diverse mature morphologies. Two consequences of the second level of ontogenetic comparisons were: 1) characters typically used in the classification of the family were recoded, and 2) ontogenetic characters, observable only at discreet developmental stages, were discovered.

In an alternate analysis of 55 taxa, characters with missing data (pollen characters) among Grevilleoideae were excluded and six most-parsimonious trees resulted. The phylogeny illustrates that two of the currently accepted subfamilies, Persoonioideae and Proteoideae, are not monophyletic. In addition, most of the tribes and subtribes (sensu Johnson and Briggs) are also not monophyletic.

The ontogenetic and phylogenetic analyses of proteaceous taxa presented here clarify the developmental events responsible for floral diversity in Proteaceae. Descriptions of the developmental processes involved in the morphological diversity of the family are provided and are supported by phylogenetic analyses. Hypotheses of transformation are deeply entrenched in an ontogenetic framework, thus providing empirical and testable questions. To attempt additional clarification of morphological and phylogenetic diversity among proteaceous taxa, additional ontogenetic comparisons of taxa not sampled here are needed, as well as field work on life-history traits like growth and form-architecture, pollination syndromes, and fruit dispersal. However, the taxa included in this study encompass the wide diversity represented in the five subfamilies of Proteaceae.

VITA

Andrew William Douglas was born in New York, New York, U.S.A on the 26th day of October in 1964 to Flora Brooks Douglas and Donald Bruce Douglas. Andrew graduated from Lake Forest High School in 1982 and moved to Wooster, Ohio where he attended college at the College of Wooster. As an undergraduate, he majored in Biology and completed an independent study thesis titled, "The effects of xanthine oxidase on plasmalogen levels in the aortic and myocardial tissues of juvenile rats." He minored in philosophy and economics and graduated with a B.A. in 1986. After a brief interim as a corporate loan officer in a Chicago bank and a retail specialist at a video store in Lake Forest, Andrew moved to Bloomington, Illinois where he pursued graduate studies at Illinois State University, Normal. Under the guidance of Dr. Joseph Armstrong, Andrew studied floral ontogenetic diversity in relation to pollination syndromes of the North American Louseworts (Pedicularis: Scrophulariaceae). Andrew received a Master of Science Degree in Biology in December, 1989 and received the "Outstanding dissertation or thesis of Illinois State University" award for his research and the Jack A. Ward award for his service to fellow graduate students.

In August of 1989, Andrew was accepted into the graduate program in the Botany Department at Louisiana State University. Under the guidance of Dr. Shirley C. Tucker and as her research assistant, Andrew has co-authored several papers, received a National Science Foundation grant for dissertation research, presented talks on his research at five national meetings, been an invited speaker at two symposia, six universities and one high school; been recipient of two awards for reports given at Botanical Society of America meetings (the Esau Award for structural and

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Andrew William Douglas

Major Field: Botany

Title of Dissertation: Evolutionary Patterns in Proteaceae Based on Comparative Floral and Inflorescence Ontogenies

Approved:

Shirley C. Tucker
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

July 29, 1994